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ROBERT STENHOUSE WILLIAMS

AN APPRECIATION

THE world of agricultural research learned with very real regret of the sudden death of Dr Robert Stenhouse Williams, M.B., C.M., D.Sc., D.P.H., Director of the National Institute for Research in Dairying, which occurred on February 2, 1932.

The son of a medical man, Stenhouse Williams was born in 1871, and qualified in medicine at Edinburgh in 1896. He also held the Cambridge D.P.H. After qualifying, he studied at the Pasteur Institute in order to perfect his knowledge of bacteriology and immunology.

From the first he was interested in hygiene and public health, and he held several hospital appointments in Liverpool and district, as well as teaching posts in the University. In 1912 he was working in the Serum Department of the Liverpool Incorporated Institute of Comparative Pathology.

At this time the Ministry of Agriculture founded the Dairy Research Institute at Reading. Stenhouse Williams seized the opportunity of entering a field which, so far as this country was concerned, was almost unexplored, and accepted the position of Research Bacteriologist. He began at once to make a study of the condition of the milk supply from the bacteriological point of view, and quickly confirmed the general view that improvements were essential. He held, however, that, in order to convince the milk producer and retailer of their shortcomings, it was necessary to bring definite evidence before their eyes, and he started a long series of experiments which showed the various sources of contamination of milk. At the same time he devised practical methods of overcoming the difficulties. These were adopted by a small band of enthusiasts who, under Stenhouse Williams' guidance, followed the lead of Mr Wilfred Buckley—whose example and help in the early years were of the greatest value to the movement. The success of this small band of pioneers soon led to the adoption of clean milk methods by others.

In 1920 the first Clean Milk Competition was held in Essex with Stenhouse Williams and the writer as judges. The value of such competitions was quickly appreciated and the Ministry of Agriculture was persuaded to give its support to the County Clean Milk Competitions, which have since played so large a part in the general improvement of our milk supplies. The creation of these competitions led directly to the establishment of the Dairy Bacteriology Advisory Service, which has since proved to be invaluable.

It was largely due to Stenhouse Williams' early work at Reading that it became possible for the Ministry of Health to sanction and extend the grading of milk. The establishment of graded milk gave Stenhouse Williams the

liveliest satisfaction, not only because a bacteriological standard was imposed, but because, for the highest grades, the cows were required to pass the tuberculin test. Very soon many towns had a small but valuable supply of clean safe milk, and nuclei of healthy herds were created all over the country.

With this weapon in his hand, Stenhouse Williams entered enthusiastically into his campaign for the preservation of an open door for clean safe raw milk. It has frequently been said that Stenhouse Williams was an opponent of pasteurisation. This is not true. He recognised as clearly as those who did not agree with him that the larger towns could not be provided with a good milk supply without pasteurisation. But he held that, until we know what changes, if any, are brought about in the nutritive value of milk by pasteurisation, pasteurised milk ought not to be compulsorily imposed on the consumer. He emphasised the fact that, although these possible changes may be difficult to demonstrate, there is no good reason for stating that they do not exist to a significant extent. In adopting this attitude he was merely affirming his belief that the young and vulnerable part of the population ought not to be allowed to run the risk of general malnutrition, which might ultimately affect profoundly the health of the whole population.

Stenhouse Williams' propaganda for a clean milk supply so far succeeded that an increasing number of wholesale firms have recognised the value of clean milk—for whatever purpose it may be destined—and have instituted control schemes, to the benefit of their suppliers and themselves. He was, moreover, able to prove that it is perfectly feasible to market raw milk in large cities like London, and to deliver it to the consumer in an entirely satisfactory condition. His work was not confined to raw milk, but extended to large numbers of pasteurising and sterilising plants, where he was able to give valuable help in eliminating sources of contamination.

Stenhouse Williams was indefatigable in his work for the dairy industry. He was an active member of the Dairy Committee of the Royal Agricultural Society of England, and of the Council of the British Dairy Farmers' Association, whose journal he edited and raised to a high pitch of excellence. He founded the Society of Agricultural Bacteriologists, and took a keen interest in the People's League of Health.

One of the things which pleased him most was the founding, under the auspices of the Empire Marketing Board, of the *Journal of Dairy Research*, of which he was the first Editor. For years he had felt the need for a journal devoted to the very specialised work of the dairy industry, and forming a link between workers in all parts of the Empire, whose interests he had very much at heart. As the Chairman of the Dairy Research Committee of the Empire Marketing Board he had the latter object closely before him.

In spite of the fact that his time was so much occupied in raising funds for his Institute, and fulfilling his outside duties, he found time, before his appointment as Director, to do a good deal of fundamental research work. Several papers on "The viability of the bovine tubercle bacillus under natural

conditions," and on "The causes of discoloration in cheese," bear testimony to this aspect of his activities.

Stenhouse Williams' loyalty to his friends and his Institute was a by-word among those who knew him well. He always refused to accept credit for any work which was not his own, and his capacity for encouraging his fellow-workers, both in the Institute and outside it, was remarkable. His deeds of kindliness were many, but were heard of only by accident. To those who did not agree with him he presented an uncompromising front, but his opponents always admired his steadfastness of purpose.

He was a sturdy individualist and insisted on playing his own hand. At bridge he would not allow dummy to play even the last card.

His name will endure in the memory as one who fought steadfastly for the great industry to which he devoted his life and as one who was ever jealous for its fair name. The National Institute for Research in Dairying which, very largely as a result of his untiring efforts, has reached its present proportions from very small beginnings, remains a monument to his energy and self-sacrifice.

A. T. R. M.

35. THE CHEMICAL CONSTITUTION OF THE PROTEINS

By R. H. A. PLIMMER, D.Sc.

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Introduction.

The nitrogenous substances or albuminoids, of which animal tissues for the most part consist, and which are also found in the seeds, leaves and other parts of plants, were originally believed to be composed of one and the same organic substance called protein by Mulder about a century ago (from πρωτεύω, "I am the first").

Though the idea of one substance as the basis of albuminoid matter has become untenable, still it is not altogether erroneous, since proteins have been found to be composed of a single class of organic compounds—the amino acids—which are united together in a fairly definite manner. The variety of the proteins arises from the number and proportion of the different amino acids in the molecule; sometimes all may be present, forming the so-called complete protein; sometimes one or more of the amino acids may be absent: these are the incomplete proteins. Sometimes there may be one or more amino acids present in large amounts whilst others are present in very small amounts: these may be called disproportionate proteins. The simplest simile representing proteins is that of boxes of mixed biscuits. Some boxes may contain all varieties in suitable proportions, others may not have all the varieties, and others may have a preponderance of a few kinds with very few of other kinds.

The similarity of proteins is shown by their elementary composition, which has the following variations:

C	51–55 %	N	15·0–19·0 %
H	7 %	S	0·4– 2·5 %
O	20–30 %	P	0·1– 0·8 %

from which a complex formula, such as

$$C_{726}H_{1174}O_{214}N_{124}S_3$$

can be calculated.

A molecular weight of about 16,000 follows from such an empirical formula, but in many cases a molecular weight of 34,000 is deduced from direct determinations. These few data emphasise the complexity of the protein molecule and indicate the difficulties of investigation.

The amino acids.

Of the various methods used in the study of the chemistry of the proteins, only that of hydrolysis has helped in the elucidation of their structure. The result of hydrolysis is a complex mixture of the amino acids which must be separated into individual substances, and which have then to be characterised and identified. At first, the separation could only be accomplished by the fractional crystallisation of the amino acids, or of their copper, silver and other salts. The isolation of an amino acid was then only accomplished if it were present in large amount; the remainder of the material was represented by uncrystallisable syrups of unknown nature. Every new method that has been employed has led to the discovery of new amino acids. The use of phosphotungstic acid led to the discovery of three basic amino acids present in practically all proteins. The preparation and study of the esters of the simple mono-amino acids was most fruitful in the isolation of these compounds and showed the presence of several unknown ones. The use of butyl alcohol as a solvent for the simpler amino acids made possible their complete removal from the mixture and the discovery of yet another compound. The names of Drechsel and Kossel will always be associated with the study of the basic amino acids, the name of Emil Fischer with the esters and isolation of the simple amino acids, that of Dakin with the butyl alcohol method. Coupled with the names of these workers there must be that of Hopkins who discovered tryptophan, one of the most important constituents of the protein molecule. The amino acids which have been discovered to be present in proteins are given in Table I, together with the names of their discoverers and the date. The period of time covered is 110 years, but our more exact knowledge starts from about 1900. There are indications that other amino acids have still to be found.

Besides the isolation of the amino acids from proteins, their exact constitution has had to be ascertained. In the early days this problem presented considerable difficulty, and it often happened that many years intervened between the discovery of an amino acid and the final proof of its constitution by synthesis. Now, the discovery of an amino acid is followed almost immediately by its synthesis. The comparison of the date of discovery and the synthesis is included in Table I.

The amino acids, with the exception of the simplest, glycine, are optically active and only one of the stereo-isomers occurs in the proteins. The synthetical compound is usually a mixture of the optically active forms. The synthesis is complete only when the optical isomers have been separated. This part of the subject has been accomplished with almost complete success, only two or three of the synthetic amino acids still require separation into their stereo-isomers. Table I also shows the natural form and the success achieved in its preparation.

Table I.

	Discovered		Synthesised			Natural form prepared	
	In	By	In	By		In	By
Glycine	1820	Braconnot	1858	Perkin and Duppa	—	—	—
Alanine	1879 1888	Schützenberger Weyl	1850	Strecker	*d*-	1899	Fischer
Valine	1901	Fischer	1866	Fittig and Clark	*d*-	1906	Fischer
Leucine	1819	Proust	1855 1885	Limpricht Schulze and Likiernik	*l*-	1900	Fischer
Isoleucine	1903	F. Ehrlich	1905	Bouveault and Locquin	*d*-	1907	Locquin
Norleucine	1901 1912	Thudichum Abderhalden and Weil	1893	Schulze and Likiernik	*d*-	1901	Fischer and Hagenbach
Aspartic acid	1827 1868	Plisson Ritthausen	1850	Dessaignes	*l*-	1887	Piutti
Glutamic acid	1866	Ritthausen	1890	Wolff	*d*-	1899	Fischer
Serine	1865	Cramer	1902	Fischer and Leuchs	*l*-	1906	Fischer and Jacobs
Hydroxyglutamic acid	1918	Dakin	1919	Dakin	*d*-	—	—
Phenylalanine	1881	Schulze and Barbieri	1883	Erlenmeyer and Lipp	*l*-	1907	Fischer and Scholler
Tyrosine	1846	Liebig	1883	Erlenmeyer and Lipp	*l*-	1900	Fischer
Tryptophan	1901	Hopkins and Cole	1907	Ellinger and Flamand	*l*-	—	—
Cystine	1899	Mörner	1903	Erlenmeyer	*l*-	1908	Fischer and Raske
Methionine	1922	Mueller	1928	Barger and Coyne	*l*-	1931	Windus and Marvel
Lysine	1889	Drechsel	1902	Fischer and Weigert	*d*-	—	—
Arginine	1886 1895	Schulze and Steiger Hedin	1899	Schulze and Winterstein	*d*-	—	—
Histidine	1896 1896	Kossel Hedin	1911	Pyman	*l*-	1911	Pyman
Proline	1901	Fischer	1900	Willstätter	*l*-	1909	Fischer and Zemplen
Hydroxyproline	1902	Fischer	1905	Leuchs	*l*-	—	—

The structure of the amino acids.

The amino acids, with the exceptions of proline and hydroxyproline, are α-amino acids, and are most conveniently classified in eight groups:

I. Simple mono-amino acids:

Glycine, or amino-acetic acid, $CH_2(NH_2).COOH$.

Alanine, or α-aminopropionic acid, $CH_3.CH(NH_2).COOH$.

Valine, or α-amino-isovalerianic acid, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH(NH_2).COOH$.

Leucine, or α-amino-isocaproic acid, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH_2.CH(NH_2).COOH$.

Isoleucine, or α-amino-β-methyl-β-ethyl-propionic acid, $\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH.CH(NH_2).COOH$.

Norleucine, or α-amino-caproic acid, $CH_3.CH_2.CH_2.CH_2.CH(NH_2).COOH$.

II. Amino dibasic acids:

Aspartic acid, or α-aminosuccinic acid, $HOOC.CH_2.CH(NH_2).COOH$.

Glutamic acid, or α-aminoglutaric acid, $HOOC.CH_2.CH_2.CH(NH_2).COOH$.

III. Hydroxy-amino acids:

Serine, or β-hydroxy-α-aminopropionic acid, $CH_2OH.CH(NH_2).COOH$.

Hydroxyglutamic acid, or β-hydroxy-α-aminoglutaric acid,

$$HOOC.CH_2.CHOH.CH(NH_2).COOH.$$

IV. Amino acids with aromatic nucleus:

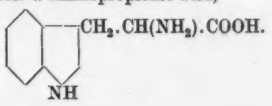
Phenylalanine, or β-phenyl-α-aminopropionic acid, $C_6H_5.CH_2.CH(NH_2).COOH$.

Tyrosine, or *p*-hydroxy-β-phenyl-α-aminopropionic acid,

$$HO.C_6H_4.CH_2.CH(NH_2).COOH.$$

V. Amino acid with indole nucleus:

Tryptophan, or β-indole α-aminopropionic acid,



VI. Sulphur containing amino acids:

Cystine, or dicysteine, or di- (β-thio-α-aminopropionic acid),

$$HOOC.CH(NH_2).CH_2.S—S.CH_2.CH(NH_2).COOH.$$

Methionine, or γ-methylthiol-α-aminobutyric acid,

$$CH_3.S.CH_2.CH_2.CH(NH_2).COOH.$$

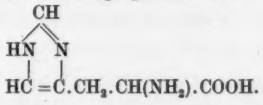
VII. Basic amino acids, or hexone bases:

Lysine, or α, ε-diaminocaproic acid, $CH_2(NH_2).CH_2.CH_2.CH_2.CH(NH_2).COOH$.

Arginine, or δ-guanidine-α-aminovalerianic acid,

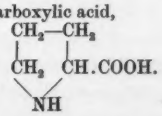
$$NH_2.C(NH).NH.CH_2.CH_2.CH_2.CH(NH_2).COOH.$$

Histidine, or β-iminazole-α-aminopropionic acid,

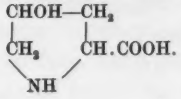


VIII. Heterocyclic compounds:

Proline, or α-pyrrolidine carboxylic acid,



Hydroxyproline, or γ-hydroxy-pyrrolidine carboxylic acid,



Other amino acids.

The amino acids above enumerated are present in all except a few proteins and consequently are in a class by themselves, as they form the background of the subject. Other amino acids have been isolated from some special proteins. Hydroxy-aminobutyric acid, hydroxyvaline, hydroxylysine and a base with eight carbon atoms, called proctoctine, have been described by Schryver and Buston as present in certain vegetable proteins, dihydroxyphenylalanine has been isolated by Guggenheim from leguminous plants.

It is interesting to notice that the iodine-containing compounds, 3, 5-diiodotyrosine and thyroxine of the thyroid gland are derivatives of tyrosine. Thyroxine has been synthesised by Harington and Barger.

Various other amino acids have been prepared by synthesis, and their characterisation must be helpful in identifying any new amino acid still undiscovered in the protein molecule.

The chemistry of the proteins is thus largely the chemistry of the amino acids, and is conveniently divided into four parts:

(1) Hydrolysis of proteins and separation of the amino acids.

(2) Synthesis and constitution of the amino acids and their separation into optical isomers.

(3) Structure of proteins, polypeptides and diketopiperazines.

(4) Relative importance of the amino acids in nutrition.

Part I. Hydrolysis of proteins and isolation of the amino acids.

Hydrolysis.

Hydrolysis of proteins is most usually carried out by boiling with acids for 15 or 24 hours or more, either with six times the quantity of 25 or 33 per cent. sulphuric acid, or with three times the quantity of concentrated hydrochloric acid, or with ten to twenty times the quantity of 20 per cent. HCl. Complete hydrolysis is more quickly effected by heating the protein, previously dissolved in the acid, for $1\frac{1}{2}$ hours in an autoclave at 150° C. with 3*N* acid. Completion of the hydrolysis is ascertained by performing the biuret reaction which must be negative. It is better to test completion by estimating the amino nitrogen by the Van Slyke method at intervals of 5 or 6 hours.

The choice of hydrolysing agent depends upon the products that it is wished to isolate. A complete isolation of all the amino acids has seldom been carried out with one lot of material. Hydrolysis with sulphuric acid has generally been preferred, as it can be subsequently removed as barium sulphate. It is used for the isolation of the basic amino acids, and for butyl alcohol extraction. Hydrolysis with hydrochloric acid is preferred for isolation of the mono-amino acids.

Hydrolysis with alkali is not often performed since the alkali destroys arginine and cystine and racemises the other amino acids. Hydrolysis with enzymes is now only used for the isolation of tryptophan, which is destroyed during hydrolysis with acids.

The isolation of the amino acids.

It is impossible in this monograph to describe in detail the various processes used in the isolation of the amino acids. A short outline will show that numerous manipulations are necessary and that several months elapse during the progress of an experiment.

A. *Extraction with butyl alcohol.*

Dakin described this method in 1918, using sulphuric acid for hydrolysis. This procedure as the first stage has been tried with casein, gliadin, glutenin, zein, edestin and gelatin, and is likely to be adopted as a standard. It worked well for casein, but did not prove so easy for gelatin. It has the great disadvantage that the extraction must be continued for several days and may still be incomplete.

Butyl alcohol extracts the mono-basic mono-amino acids together with proline and hydroxyproline. From this mixture, after removal of the butyl alcohol, proline is separated by solution in ethyl alcohol. The remaining amino acids are then separated by the ester method of Fischer (below).

The mono-amino dibasic acids and the basic acids are not dissolved by the butyl alcohol. The basic amino acids are separated by precipitation with phosphotungstic acid (below) and the remaining three dibasic acids are separated by precipitating the glutamic acid as hydrochloride by passing in dry hydrogen chloride. Aspartic acid is best removed as lead salt, and the hydroxyglutamic acid is isolated by precipitation as silver salt in baryta solution.

B. *The ester method for separating the mono-amino acids.*

Amongst the mono-amino acids tyrosine and cystine are almost insoluble in water. Cystine is usually only obtainable from the keratin group of proteins. The neutralised solution of the amino acids will generally deposit the greater part of the tyrosine, and in the case of a butyl alcohol extraction it remains insoluble on dissolving the amino acids insoluble in alcohol in water.

The solution of the amino acids is evaporated and the dry, or almost dry, residue is suspended in absolute alcohol and esterified by passing in dry hydrogen chloride. The ester hydrochlorides are formed. The operation of esterification should be repeated. Glycine ester hydrochloride will now crystallise out if the protein contain a large proportion. The solution is concentrated, treated with excess of solid baryta according to the method of Levene and Van Slyke and the esters so liberated are dissolved in ether. The ethereal solution is dried and the esters are separated by fractional distillation *in vacuo*. The esters are hydrolysed and the amino acids isolated by crystallisation. The ester fraction containing valine, leucine and isoleucine presents

the greatest difficulty; its separation has seldom been properly effected. The mixture is generally returned as leucine.

C. *The separation of the basic amino acids.*

In the case of a butyl alcohol extraction the basic amino acids are obtained as phosphotungstates. They are obtained by decomposing with baryta, and separated by the silver-baryta method of Kossel and Patten. This method has been carefully studied by Vickery and Leavenworth. It is used directly after sulphuric acid hydrolysis. The method consists in precipitating the silver salts of arginine and histidine in presence of excess of baryta, and the lysine from the filtrate as phosphotungstate. Arginine and histidine are subsequently separated by precipitating histidine as silver salt in neutral solution, and arginine in alkaline solution.

The new method of Kossel and Gross of precipitating arginine as flavianate is likely to supersede the silver method. It is very useful for preparing arginine from gelatin as shown by Pratt.

D. *Tryptophan.*

For the isolation of tryptophan the protein is hydrolysed by the action of trypsin. The solution, filtered from tyrosine, is acidified with sulphuric acid and precipitated with mercuric sulphate. After decomposition of the precipitate with hydrogen sulphide and removal of hydrogen sulphide the solution is best extracted with butyl alcohol so as to separate tryptophan from histidine. Tryptophan crystallises from the butyl alcohol on evaporation.

The results of hydrolysis.

A large number of proteins have been analysed. A selected set of analyses is given in Table II. In most cases the amounts of amino acids only total about 60 per cent. Consequently, *the figures are not to be regarded as quantitative.* The deficit belongs mainly to the group of mono-amino acids. The figures for the basic amino acids are probably a very near representation of the actual amount in the proteins.

The data clearly show differences in the composition of the proteins of the various groups. Thus, gelatin and silk fibroin contain large amounts of glycine. Salmine and other protamines are characterised by consisting almost entirely of basic amino acids. The gliadins contain a very large amount of glutamic acid and have also a high content of proline. The vegetable globulins have a large amount of arginine. The keratins contain most cystine. Haemoglobin has the largest amount of histidine.

The figures show the differentiation of proteins into complete, incomplete and disproportionate. These differences are of importance in considering the values of proteins in nutrition.

Except the most recent analyses of casein, gelatin and a few other proteins, full information of the data is given in the author's *Chemical Constitution of the Proteins* (Longmans, Green and Co., 1918).

Table II. *Compositions of proteins.*

	Casein milk	Vitellin egg-yolk	Albumin milk	Albumin egg-white	Globulin serum	Haemoglobin	Muscle ox	Muscle fish	Gelatin	Silk fibroin	Horn keratin
Glycine	0	0	0	0	3·5	–	2·1	0	25·5	36·0	0·4
Alanine	1·5	0·8	2·5	8·4	2·2	4·2	3·7	?	8·7	21·0	1·2
Valine	7·2	1·9	0·9	–	–	–	0·8	0·8	0	0	5·7
Leucine	9·4	9·9	19·4	15·2	18·7	29·0	11·7	10·4	7·1	1·5	18·3
Isoleucine	–	–	–	–	–	–	–	–	–	–	–
Phenylalanine	3·2	2·6	2·4	5·2	3·8	4·2	3·2	3·1	1·4	1·5	3·0
Tyrosine	4·5	3·4	0·9	1·0	2·5	1·3	2·2	2·4	0	10·5	4·6
Tryptophan	1·7	+	.	.	+	+	+	+	0	–	–
Cystine	+	.	–	0·4	0·9	0·3	–	–	–	–	6·8
Aspartic acid	4·1	2·2	1·0	1·7	2·5	4·4	4·5	2·8	3·4	+	2·5
Glutamic acid	21·6	13·0	10·1	3·5	8·5	1·7	15·5	10·1	5·8	0	15·0
Serine	0·5	–	–	–	–	0·6	–	–	0·4	1·6	0·7
Hydroxyglutamic acid	10·5	–	–	–	–	–	–	–	0	–	–
Arginine	3·8	7·5	3·2	2·1	–	5·4	7·5	6·4	8·2	1·0	2·3
Histidine	2·5	1·9	2·1	–	–	11·0	1·8	2·6	0·9	+	–
Lysine	6·0	4·8	9·2	2·3	–	4·3	7·6	7·5	5·9	+	+
Proline	8·0	4·2	4·0	1·1	2·8	2·3	5·8	3·2	9·5	0·3	3·6
Hydroxyproline	0·3	–	–	–	–	1·0	–	–	14·1	–	–
Ammonia	1·6	1·3	1·3	–	–	–	1·1	1·4	0·4	–	–
Total	86·4	53·5	57·0	40·9	45·4	69·7	67·5	50·7	91·3	73·4	64·1

Table II (*continued*).

	Wool keratin	Salmon salmine	Gliadin wheat	Gliadin rye	Zein maize	Glutenin wheat	Gluten wheat	Albumin wheat leucosin	Edestin hemp seed	Legumin pea	Almond amandin
Glycine	0·6	–	0	0·1	0	0·9	0·4	0·9	3·8	0·4	0·5
Alanine	4·4	–	2·0	1·3	9·8	4·7	0·3	4·5	3·6	2·1	1·4
Valine	2·8	4·3	3·4	–	1·9	0·2	–	0·2	+	–	0·2
Leucine	11·5	–	6·6	6·3	19·6	6·0	4·1	11·3	20·9	8·0	4·5
Isoleucine	–	–	–	–	–	–	–	–	–	–	–
Phenylalanine	0	–	2·4	2·7	6·6	2·0	1·0	3·8	2·4	3·8	2·5
Tyrosine	2·9	–	1·2	1·2	3·6	4·3	1·9	3·3	2·1	1·6	1·1
Tryptophan	–	–	1·0	+	0	+	–	+	+	–	+
Cystine	7·3	–	0·5	–	–	0·02	–	–	–	–	–
Aspartic acid	2·3	–	0·6	0·3	1·7	0·9	0·7	3·4	4·5	5·3	5·4
Glutamic acid	12·9	–	43·7	33·8	26·2	23·4	24·0	6·7	6·3	17·0	23·2
Serine	0·1	7·8	0·2	0·1	1·0	0·7	–	–	0·3	0·5	–
Hydroxyglutamic acid	–	–	–	–	–	–	–	–	0	–	–
Arginine	–	87·4	3·2	2·2	1·6	4·7	4·4	5·9	11·7	11·7	11·9
Histidine	–	0	0·6	0·4	0·8	1·8	1·2	2·8	1·1	2·4	1·6
Lysine	–	0	0·2	0	0	1·9	2·2	2·8	1·0	5·0	0·7
Proline	4·4	11·0	13·2	9·8	9·0	4·2	4·0	3·2	1·7	3·2	2·5
Hydroxyproline	–	–	–	–	–	–	–	–	2·0	–	–
Ammonia	–	–	5·2	5·1	3·6	4·0	2·5	1·4	–	2·1	3·7
Total	49·2	110·5	84·0	63·3	85·4	59·72	46·7	50·2	61·4	63·1	59·2

Estimation of tyrosine, tryptophan and cystine.

The three compounds, tyrosine, tryptophan and cystine on account of the presence of special groupings in their molecules give distinctive colour reactions by means of which their presence in proteins can be detected. The

colour reactions have been studied and developed and it is possible to estimate the amounts of these amino acids in proteins with fair accuracy.

Tyrosine and tryptophan.

In 1912 Folin and Denis introduced a method for estimating tyrosine, depending on the blue colour which it gives with a phosphotungstic-phosphomolybdic reagent. The high values so obtained were subsequently shown to be due to decomposition products of tryptophan. Folin and Looney modified the method in 1922 and criticisms were answered by Looney in 1926, who gave some amended data. In the modified method both tyrosine and tryptophan were estimated.

Cystine.

Folin and Looney at the same time described a colorimetric method for estimating cystine, which depends upon the reduction of cystine to cysteine, which gives a blue colour with a special (uric acid) reagent.

It is pointed out that the values obtained by these methods for tyrosine, tryptophan and cystine give molecular weights for the proteins which are very close to the molecular weights deduced by physico-chemical methods by Cohn, Hendry and Prentiss. It is interesting that the values for tryptophan correspond in the main with those of May and Rose (below).

Table III gives some of the data.

Table III.

	Tyrosine %	Tryptophan %	Cystine %
Gliadin	3·04	1·10	2·40
Glutenin	4·56	1·64	1·80
Edestin	4·58	1·52	0·84
Egg albumin	4·10	1·26	0·81
Gelatin	0	0	0·17
Casein	5·32	1·54	0·25
Wool	5·52	1·45	7·8
Horn	5·28	1·43	6·67
Serum globulin	6·7	2·28	.
Fibrin	6·5	2·90	3·5
Zein	5·66	0	0·75

Tryptophan.

Tryptophan gives colour reactions with various aldehydes in acid solution. The colour reaction with glyoxylic acid has been used for its detection for many years and has been applied for its estimation. Other aldehydes which give colour reactions are formaldehyde, vanillin and *p*-dimethylaminobenzaldehyde. Of these, that with *p*-dimethylaminobenzaldehyde has been adapted to a most convenient method of estimation by May and Rose. An intense blue colour is produced and it is matched with the blue colour given by casein under the same conditions, which is taken as the standard. May and Rose took the figure of 1·5 per cent. of tryptophan in casein and Jones, Gersdorff

and Moeller the figure of 2·2 per cent. as the amount in casein. The latter figure was considered more correct, since 1·7 per cent. of tryptophan has actually been isolated from casein, and other colorimetric methods indicate from 2·0 to 2·2 per cent.

A selection of the values for tryptophan in proteins by this method is given in Table IV. May and Rose's figures in column 1 have been multiplied by 1·47 to give the same standard of 2·2 per cent. used by Jones, Gersdorff and Moeller and are shown in column 2. The figures obtained by Jones, Gersdorff and Moeller are in column 3.

Table IV. *Percentage amounts of tryptophan.*

	May and Rose	× 1·47	Jones, Gersdorff and Moeller
Casein	1·5	2·2	.
Lactalbumin	2·4	3·5	2·69
Gliadin	1·05	1·5	1·01
Glutenin	1·80	2·6	1·72
Edestin	1·5	2·2	2·48
Glycinin (soya)	1·65	2·4	1·66
Ovovitellin	1·74	2·6	2·42
Ovalbumin	1·11	1·6	.
Phaseolin	0·80	1·2	.
Maize, gluten	1·08	1·6	.
Legumin, vetch	1·05	1·5	.
Legumin, pea	.	.	1·76
Vicilin	.	.	0·15
Zein	0	0	.
Gliadin, rye	.	.	0·75
Hordein, barley	.	.	1·05
Oat, gliadin	.	.	0
Gelatin	0	.	.
Ox muscle	.	.	1·25
Fish muscle	.	.	1·25
Fibrin	.	.	4·4
Spinach, protein	.	.	1·85
Alfalfa, protein	.	.	2·86
Cowpea, vignin	.	.	1·65
Buckwheat, globulin	.	.	2·69

It will be noticed that there are many discrepancies in the values obtained by these two groups of workers. The explanation may be due to the different preparations of the proteins that were used. Jones, Gersdorff and Moeller have shown that different values are obtained for different preparations of the same protein and consider that the divergencies are due rather to the sample of protein than to errors in the method. The method is, however, liable to give the different values. Boyd has shown that the blue colour is hindered in its development by the presence of reducing substances in the solution and varies with the intensity of the light whilst the solution stands before making the comparisons. The discrepancies may also be due to incomplete hydrolysis of the protein. It is well known that the different proteins are not hydrolysed at the same rate. The values by the method of Folin and Looney would appear preferable and more accurate.

Analysis of proteins by distribution of nitrogen.

The constitutions of the amino acids show that they belong to several different groups. On hydrolysis, besides the amino acids, small quantities of ammonia are liberated from amide groupings, and a small quantity of so-called humin nitrogen, derived from the decomposition of certain amino acids, especially tryptophan, is formed. The amino acids belong to two main groups, the mono-amino acids with which proline and hydroxyproline are associated, and the basic or di-amino acids with which cystine is included. These two main groups are separated by the precipitation of the di-amino acid group with phosphotungstic acid. The compounds in the two groups can be subdivided according to their behaviour towards nitrous acid. Only those compounds react which contain amino groupings according to the equation:

$$\begin{matrix} R.CH.NH_2 \\ | \\ COOH \end{matrix} + ONOH = \begin{matrix} R.CH.OH \\ | \\ COOH \end{matrix} + N_2 + H_2O.$$

All the mono-amino acids react, but proline and hydroxyproline do not. All the di-amino acids react, but they can be differentiated by other reactions. Thus, the guanidine grouping of arginine is decomposed by boiling with alkali with evolution of ammonia; cystine is characterised by its sulphur content which can be determined. Van Slyke first pointed out these differences and described the method of determining seven values out of a possible nineteen or twenty, and also devised a most convenient apparatus for estimating the amino nitrogen.

The estimations are the following:

(1) Amide nitrogen.

(2) Humin nitrogen.

(3) Total di-amino or basic nitrogen:

(*a*) Arginine nitrogen. Half of its nitrogen is evolved by boiling with alkali, hence its amount. One-quarter of its nitrogen is present as amino nitrogen.

(*b*) Histidine nitrogen. One-third of its nitrogen is present as amino nitrogen.

(*c*) Lysine nitrogen. The whole of its nitrogen is present as amino nitrogen.

(*d*) Cystine nitrogen. The whole of its nitrogen is present as amino nitrogen, and is calculated from the sulphur content.

The non-amino nitrogen of these compounds belongs to the arginine and the histidine. Hence, from determinations of the total nitrogen, arginine nitrogen, amino nitrogen and sulphur the amounts of the four amino acids can be ascertained:

Arginine nitrogen is twice the nitrogen evolved on boiling with alkali. The non-amino nitrogen is divided between the arginine and histidine:

Histidine nitrogen is 3/2 (amino nitrogen—3/4 arginine nitrogen) or 1·5 amino nitrogen—1·125 arginine nitrogen.

Cystine nitrogen is calculated from the sulphur content.

Lysine nitrogen is total nitrogen—(arginine nitrogen + histidine nitrogen + cystine nitrogen).

(4) Total mono-amino nitrogen:

(*a*) Amino nitrogen from all the simple amino acids.

(*b*) Non-amino nitrogen from proline and hydroxyproline.

The procedure of the method is very simple and can be rapidly carried out. The details are given in the original papers by Van Slyke and they have been thoroughly examined by Plimmer and Rosedale. The sulphur estimation has been studied by Plimmer and Lowndes.

This method of nitrogen distribution has been widely adopted in the examination of proteins of all sources. It is essential that the proteins be isolated from their natural sources, since the presence of other nitrogenous substances in animal products unless removed will give erroneous results, and the hydrolysis of vegetable proteins in presence of carbohydrate gives too high values for humin nitrogen from decomposition of amino acids heated with acid under these conditions. Foodstuffs cannot therefore be analysed by this method.

The method unfortunately gives little information about the mono-amino acids and does not differentiate proline and hydroxyproline. It gives values for the basic or di-amino acids which are generally higher than those obtained by the Kossel-Patten method. They are regarded as the more accurate.

Of the very large number of proteins which have been analysed by this method a selection has been made and the data are given in Table V. The figures are in terms of nitrogen. The various workers have usually not converted the data into amounts of the amino acids in the proteins. The conversion can be made by using the nitrogen value of the protein to calculate its amount in place of 100, and by calculating the di-amino acids from the figure given and its amount in the particular compounds.

Table V. *Nitrogen distribution of some proteins.*

	Amide	Humin	Cystine	Arginine	Histidine	Lysine	Mono-amino	Mono-non-amino	Total
Casein	10·27	1·28	0·20	7·41	6·21	10·30	55·81	7·13	98·61
Lactalbumin	7·72	1·65	2·34	7·27	4·20	13·08	60·91	2·03	99·20
Egg-yolk	9·0	2·4	.	14·5	3·1	9·4	60·6	1·6	100·6
Egg-white	9·2	2·0	.	11·7	0·2	10·1	66·0	1·6	100·9
Gelatin	2·25	0·07	0	14·70	4·48	6·32	55·8	14·9	99·02
Haemoglobin	5·24	3·60	0	7·70	12·70	10·90	57·00	2·9	100·04
Pig muscle	8·0	6·8	.	8·1	1·9	8·4	65·7		98·9
Fish muscle, mackerel	8	1	.	12	5	11	56	6	99
Edestin	9·99	1·98	1·49	27·05	5·75	3·86	47·55	1·70	99·37
Gliadin, wheat	25·52	0·86	1·25	5·71	5·20	0·75	51·98	8·50	99·77
Prolamin, wheat bran	23·0	1·18	1·23	9·12	1·45	3·02	56·13	5·09	100·22
Albumin, wheat bran	7·66	4·31	2·10	20·70	4·45	5·54	56·28	0·80	100·84

Part II. Syntheses and constitution of the amino acids.

Every amino acid which has been isolated from proteins and is considered to be a definite constituent has been prepared by synthesis to prove its constitution. In most instances the synthesis has been effected since its discovery amongst the products of hydrolysis of proteins. Only alanine and proline had been synthesised previously. The isolation of a new amino acid has in fact given the stimulus to its synthesis by regular systematic methods. The synthesis must be regarded as an important part of the subject of the chemistry of the proteins.

Though there are several general methods for the synthesis of amino acids, it has often been found necessary to modify the process on account of various difficulties which have arisen, such as inaccessibility of the starting material, or poor yields of product. The preparation of the amino acid by synthesis is often the best method for its preparation for its use in experiments in metabolism or in synthesising polypeptides. A short summary of the various methods will present numerous points of interest and will show how difficulties of synthesis have been overcome.

The four most general methods of synthesis are:

(1) The action of ammonia upon the corresponding halogen substituted fatty acid.

(2) The addition of hydrogen cyanide and ammonia to the lower aldehyde, followed by the hydrolysis of the nitrile group.

(3) The synthesis by means of ethyl hippurate and reduction of the unsaturated condensation compound.

(4) The reduction of the corresponding nitroso or imino compound.

Method 1.

This method has been used in the synthesis of:

(1) glycine from chloracetic acid;
(2) alanine from α-chloro- or α-bromopropionic acid;
(3) valine from α-bromo-isovaleric acid;
(4) leucine from α-bromo-isocaproic acid;
(5) norleucine from α-bromocaproic acid.

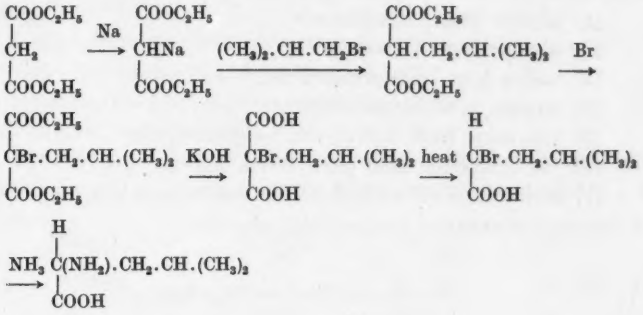
It is only of value for the preparation of glycine:

$$\begin{matrix} CH_2Cl \\ | \\ COOH \end{matrix} + NH_3 = \begin{matrix} CH_2NH_2 \\ | \\ COOH \end{matrix} + HCl$$

Its modification by the use of malonic ester is the best way for preparing:

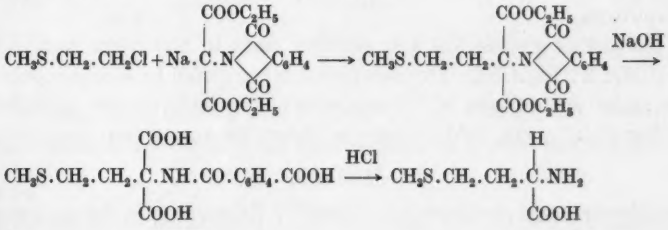
(1) leucine from isobutyl bromide;
(2) isoleucine from sec.-butyl iodide;
(3) phenylalanine from benzyl bromide;
(4) proline from trimethylene dibromide;
(5) hydroxyproline from epichlorhydrin;
(6) histidine from chloromethyl-glyoxaline.

There are many steps in the synthesis, which may be illustrated by that of leucine (Fischer and Schmitz):



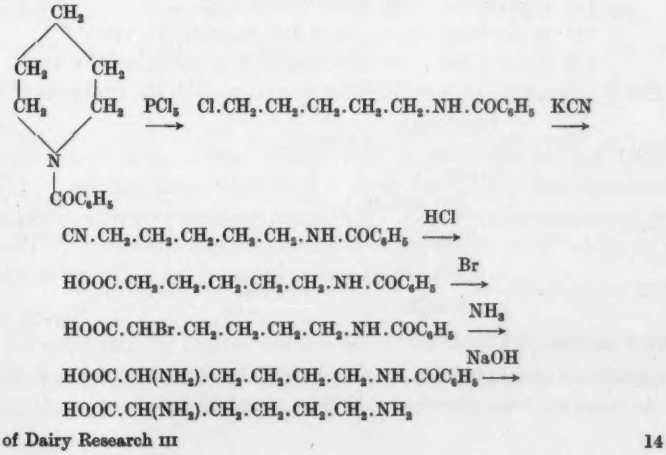
Methionine.

A modification of the malonic ester method introducing potassium phthalimide, first used by Sörensen in the synthesis of phenylalanine and other amino acids, was adopted by Barger and Weichselbaum for the synthesis of methionine from α-methyl-thiol-chloro-ethane:



Lysine.

The best way of synthesising lysine is by the direct method, starting from benzoyl-piperidine (v. Braun):

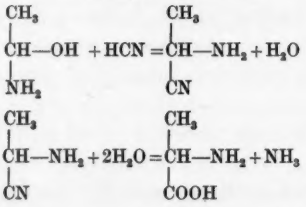


Method 2.

This method, introduced by Strecker, has been used for:

(1) glycine from formaldehyde;
(2) alanine from acetaldehyde;
(3) valine from isobutyraldehyde;
(4) leucine from isovaleraldehyde;
(5) isoleucine from methyl-ethyl-acetaldehyde;
(6) phenylalanine from phenylacetaldehyde;
(7) serine from glycollic aldehyde and from chloracetal.

It is the most convenient for preparing alanine:



The yields are good, but are improved by following the directions of Cocker and Lapworth.

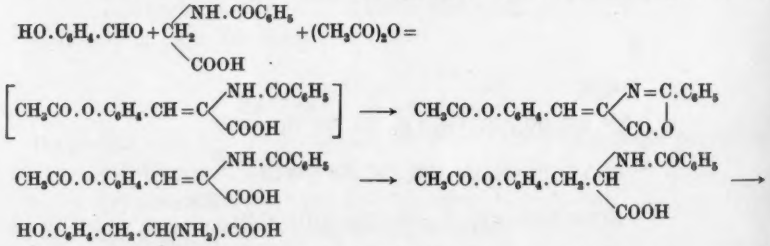
In the case of glycine, the first reaction leads to methylene amino-acetonitrile $(CH_2 : N.CH_2.CN)_3$. This compound is converted by alcoholic sulphuric acid to amino-acetonitrile, which separates out as its hydrogen sulphate, and on boiling with baryta yields glycine as shown by Anslow and King.

Method 3.

The hippuric acid synthesis introduced by Erlenmeyer is the most suitable for synthesising:

(1) phenylalanine from benzaldehyde;
(2) tyrosine from *p*-hydroxybenzaldehyde;
(3) tryptophan from β-indolylaldehyde;
(4) serine from formic ester and sodium ethylate;
(5) leucine from α-benzoylamido-β-isopropylacrylic acid.

The synthesis proceeds in the following stages, *e.g.* the synthesis of tyrosine:



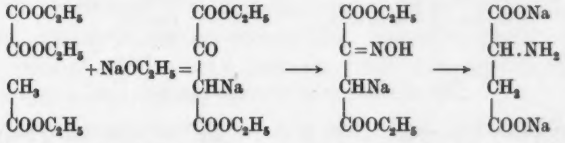
The synthesis of tryptophan by this method has been simplified and improved by the modifications introduced by Lamb and Robson.

Method 4.

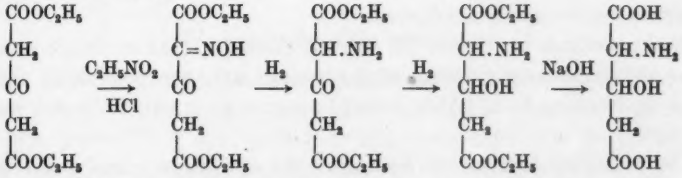
The method of reduction of the oximino compound has been used for preparing:

(1) leucine from α-oximinoisobutylacetic acid;
(2) isoleucine from sec.-butylaceto-acetic ester;
(3) aspartic acid from sodium oxalacetic ester;
(4) glutamic acid from α-nitrosoglutaric acid;
(5) hydroxyglutamic acid from isonitroso-acetone dicarboxylic ester.

It is the best method for preparing the mono-amino dibasic acids, *e.g.* aspartic acid:



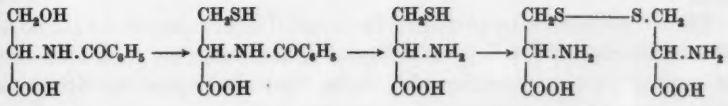
Harington and Randall succeeded in synthesising hydroxyglutamic acid in good yield as follows:



Special methods are required for the syntheses of cystine and arginine.

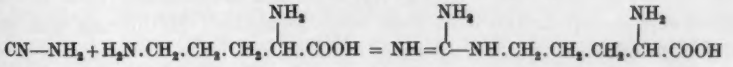
Cystine.

Cystine, or its reduction product cysteine, is prepared by the action of phosphorus pentasulphide, or barium hydrosulphide, upon benzoyl-serine. The cysteine so formed is easily oxidised by air or mild oxidising agents to cystine:



Arginine.

Arginine, like all guanidine compounds, is prepared by the action of cyanamide, upon ornithine. Ornithine is made by the Sörensen modification of the malonic ester synthesis, or more easily from benzoyl-piperidine, which is oxidised by permanganate to benzoyl-δ-aminovaleric acid, which is then converted in the same way as in the preparation of lysine:



The correctness of this reaction was finally proved by Sörensen by the reaction of cyanamide with α-monobenzoyl-ornithine.

Though many of the syntheses of the amino acids proceed through many stages, it is easier to prepare several of them in this way than by isolation from the hydrolytic products of proteins. The following lists show the best methods of preparation:

By synthesis	By isolation from proteins
Glycine	Cystine
Alanine	Tyrosine
Valine	Tryptophan
Leucine	Histidine
Isoleucine	Lysine
Norleucine	Arginine
Serine	Aspartic acid
Phenylalanine	Glutamic acid
Hydroxyglutamic acid	Proline
	Hydroxyproline

The separation of racemic amino acids.

The amino acids prepared from proteins are, with the exception of glycine, in the optically active form. The synthetical products are inactive or racemic, and consequently require separation into their optically active isomers. The real synthesis is only then achieved.

Of the methods for separating racemic mixtures, that of simple crystallisation and mechanical removal of the isomers has been possible in the case of the asparagines, from which *d*- and *l*-aspartic acids are easily prepared by hydrolysis.

The biological method of separating the optical isomers by the use of micro-organisms has been successfully employed in the isolation of *d*-leucine, *l*-glutamic acid, *d*-aspartic acid, *d*-cystine, *l*-alanine, *l*-valine, the optical antipodes of the natural amino acids from proteins.

The chemical method of crystallisation of the salts of the amino acids with optically active bases cannot be used directly on account of the small affinity for combination with these bases, and it is also not possible for the dibasic acids.

Fischer found that by preparing the benzoyl derivatives of the amino acids their acidic character was greatly increased and that they then formed with the optically active bases, strychnine, brucine, cinchonine, etc., stable salts which were soluble with greater difficulty and were more easily isolated. The benzoyl derivative is prepared by shaking the amino acid with excess of benzoyl chloride in presence of sodium carbonate. Alanine, aspartic acid, glutamic acid, tyrosine, leucine and phenylalanine were separated in this way.

Later, Fischer found that the formyl derivative was preferable to the benzoyl. It is easily prepared by heating the amino acid with anhydrous formic acid, and it is more easily hydrolysed back to the amino acid. Leucine, valine and phenylalanine were thus easily separated. Methionine was separated through the formyl compound.

In other cases, the nitrobenzoyl compound was more suited for separation, *e.g.* in the cases of serine and proline.

The combination of a dibasic amino acid with an optically active acid was used by Pyman in the case of histidine with tartaric acid.

The two amino acids, hydroxyproline and hydroxyglutamic acid, contain two asymmetric carbon atoms and will exist in four optically active forms. The separation of these stereo-isomers has not yet been effected.

Walden inversion. The complication of Walden inversion, *i.e.* change in rotation, arises in the conversion of a halogen substituted fatty acid into the amino acid: thus *d*-bromopropionic acid yields *l*-alanine, and *d*-bromovaleric acid yields *l*-valine. This change of rotation must be borne in mind in correlating the configurations of the amino acids.

Though the rotations of the amino acids may differ in being dextro or laevo, the arrangements of the groups around the asymmetric carbon atom is the same, thus *d*-alanine, *l*-serine and *l*-cystine have the same configuration though they show a difference in rotation.

This change of configuration has been worked out in several other series of organic compounds and forms a special chapter of organic chemistry, which cannot be treated in this monograph.

The properties of the amino acids.

The amino acids are colourless, crystalline substances, often with characteristic forms. With the exceptions of tyrosine and cystine they are easily soluble in water. They are insoluble in absolute alcohol, except proline, but dissolve in aqueous alcohol and acetone. All are insoluble in ether, benzene and other organic solvents. The simple amino acids are neutral, the dibasic are acid, and the di-amino are alkaline in reaction. They form salts with bases and acids, and can be made to combine with acid chlorides and other reagents producing in many cases characteristic derivatives useful for their identification. The simple amino acids may exist in the two forms

$$\begin{array}{l} R.CH.NH_2 \\ | \\ COOH \end{array} \qquad \begin{array}{l} R.CH.NH_3 \\ | \\ COO \end{array}$$

In the first they can behave as amphoteric electrolytes; in the second they can show the peculiarity of "zwitterion" formation.

PART III. STRUCTURE OF PROTEINS, POLYPEPTIDES, DIKETOPIPERAZINES.

Fischer's researches upon the mono-amino acids and Kossel's upon the basic amino acids made the general belief that proteins were composed of numerous amino acids in different proportions quite definite. The next problem was the manner of combination of the amino acids in the protein molecule. A review of all the possible ways of combination was published by Hofmeister in 1902. Combination of the amino acids through their carbon atoms was scarcely possible, and the most likely method of combination was

that of linking together of carbon atoms by a nitrogen atom. There were three possible ways:

(1) $-CH_2-NH-CH_2-$.

(2) $-CH_2-NH-CO-$.

(3) $-CH_2-NH-C(=NH)-$.

The first mode of linking is present in proline and hydroxyproline. Besides its presence in these two compounds, its occurrence to any larger extent was not likely, since the molecule would then become distinctly acid in character.

The third mode of linking is present in arginine and gives a basic character to the compound. It is not known in any other unit, and is thus not a predominant mode of linking in the protein molecule.

The second mode of linking is that of acid amide combination, and it supports the chief results of hydrolysis. The presence of the simple acid amide grouping $-CONH_2$ is indicated by the formation of ammonia. The absence of free amino groups, except to a small extent in the original protein, and their production on hydrolysis, is in accordance with the behaviour of such compounds, *e.g.* hippuric acid,

$$C_6H_5CO-NHCH_2.COOH + H_2O = C_6H_5COOH + H_2N.CH_2.COOH.$$

The acid amide linking is present in simple compounds which give the biuret reaction:

Oxamide	Biuret	Malonamide
$CO-NH_2$	$CO-NH_2$	$CO-NH_2$
$\vert$	$\vert$	$\vert$
$CO-NH_2$	NH	CH_2
	$\vert$	$\vert$
	$CO-NH_2$	$CO-NH_2$

i.e. in compounds which contain two —CO—NH groups joined together directly, or by a nitrogen atom, or by a carbon atom. The combination of three amino acids in this manner would give a compound with two such groupings:

$$H_2N.CHR.CO-NH.CHR.CO-NH.CHR.COOH.$$

The biuret reaction is a characteristic reaction of proteins and it supports such a method of combination. This kind of combination is further supported by the formation of hippuric acid and glycocholic acid in the living body. Every indication points to the acid amide form of combination as the chief and most important kind in the protein molecule. This mode of combination also lends itself to other structures by loss of water which help to explain the complicated nature of the protein molecule.

Besides the acid amide method of combination, other modes of linking may still be present in the molecule. The most probable is the combination of the hydroxyl groups of the hydroxy amino acids with the carboxyl groups of other amino acids in either ester or ether form. This kind of combination would not exist to any large extent as it would result in a basic character. There is, lastly, a possibility of an anhydride structure between two carboxyl groups, which again is not likely to be present to any large extent.

The peptides or polypeptides.

Though many attempts had been previously made to combine together two or more molecules of amino acids by simple heating, or by heating with dehydrating agents, with the formation of anhydrides of various complexity, it was not till Fischer began his systematic series of researches that any clear view of the constitution of proteins was obtained. Fischer's work proved that the acid amide form of combination, regarded by Hofmeister as the most probable, was the chief method of linking together the amino acids. Fischer termed these combinations the polypeptides, or more shortly, the peptides, a term chosen from the substance peptone, the material produced by the peptic digestion of proteins. This designation allowed of the introduction of numerals to indicate the number of amino acid radicles, *e.g.* di-, tri-, tetra-, polypeptide, and is in imitation of the nomenclature of the carbohydrates.

The synthesis of the peptides has been effected by three general methods:

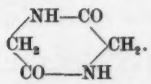
(1) By formation and hydrolysis of the anhydrides, diketopiperazines.

(2) By means of the halogen acyl compounds.

(3) By means of the acid chlorides of the amino acids and of the polypeptides.

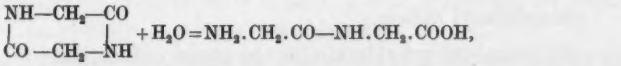
Method 1.

Glycine ester, first prepared by Curtius in 1883, was the starting-point of the researches. Curtius and Goebel found that glycine anhydride separated if the ester were allowed to stand for a few days with water. Curtius and Schulze found that the formula of the anhydride was probably



At the same time a soluble base was formed which gave the biuret reaction and was called the biuret base.

Fischer found that these anhydrides were best obtained by heating the esters of the amino acids in a sealed tube to 150–180° C. for some hours, and with Fourneau found that the anhydride was hydrolysed by boiling with concentrated hydrochloric acid into the salt of a new amino acid $C_4H_8O_3N_2$, from which they obtained the free acid by means of silver oxide. They represented its formation by the equation,



and considered that the compound was the first anhydride of two molecules of glycine. It was termed glycyl-glycine. Its ester resulted on treating the anhydride with alcoholic hydrochloric acid. Both the free acid and its ester had a great tendency to become reconverted into glycine anhydride, and both were characterised by the reactiveness of the NH_2 group.

Alanyl-alanine and leucyl-leucine were prepared in a similar way, and many of the dipeptides are most easily made by this method. They have been used in the synthesis of more complex polypeptides. The method does not lend itself to the formation of higher polypeptides, nor is it convenient for preparing mixed anhydrides containing two different amino acids. On hydrolysis of the mixed anhydride, two different dipeptides result which are very difficult to separate, thus glycyl-alanine anhydride yields glycyl-alanine and alanyl-glycine.

The dipeptides have the constitution of amino acids and can be converted into esters. These esters are not suitable for the preparation of more complex peptides. It may be noted that pentaglycyl-glycine and probably octaglycine anhydride have been made by heating the ester of diglycyl-glycine.

The anhydrides, or diketo-, or diaci-piperazines are important compounds in the consideration of the structure of the protein molecule. Several have been directly isolated from the products of hydrolysis, and this ring structure may occur in the actual constitution of the protein (see below).

Method 2.

This method of preparing polypeptides was first described by Fischer and Otto in 1903. It is similar to the combination of an acyl chloride with an amino acid, *e.g.* benzoyl chloride and glycine, and consists in the use of the halogen substituted acyl chloride, followed by the action of ammonia to replace the halogen atom, thus:

$$\underset{\text{Chloracetyl chloride}}{Cl.CH_2.COCl} + \underset{\text{Alanine}}{H_2N.CH(CH_3).COOH} = \underset{\text{Chloracetyl-alanine}}{Cl.CH_2.CO{-}HN.CH(CH_3).COOH} + HCl$$

$$Cl.CH_2.CO{-}HN.CH(CH_3).COOH + NH_3 = \underset{\text{glycyl-alanine}}{H_2N.CH_2.CO{-}HN.CH(CH_3).COOH} + HCl$$

The reaction is carried out either directly in an alkaline solution of the amino acid, or upon the ester of the amino acid in anhydrous solvents.

Numerous halogen acyl chlorides are available for the introduction of the particular amino acid grouping:

α-bromopropionyl chloride for the alanyl radicle,

α-bromisocapronyl chloride for the leucyl radicle,

α-bromohydrocinnamyl chloride or phenyl-bromopropionyl chloride for the phenylalanyl radicle,

α-δ-dibromovaleryl chloride for the prolyl radicle, etc.

Several of these halogen acyl chlorides are obtainable in the optically active form, so that it is possible to synthesise dipeptides with the natural form of the amino acid. In these syntheses the phenomenon of Walden inversion, or change in rotation during the action of ammonia, must be considered.

Since the dipeptide is again an amino acid in its constitution, the reaction

can be extended so as to produce a tripeptide, and from the tripeptide a tetrapeptide can be made, and so on to penta- and polypeptides:

$$\underset{\text{Bromisocapronyl chloride}}{Br.CH(C_4H_9)COCl} + \underset{\text{Glycyl-alanine}}{H_2N.CH_2.CO—HN.CH(CH_3).COOH} \longrightarrow$$

$$Br.CH(C_4H_9).CO—HN.CH_2CO—HN.CH(CH_3).COOH \longrightarrow$$
Bromisocapronyl-glycyl-alanine

$$H_2N.CH(C_4H_9).CO—HN.CH_2.CO—HN.CH(CH_3).COOH$$
Leucyl-glycyl-alanine
Alanyl-leucyl-glycyl-alanine
Leucyl-alanyl-leucyl-glycyl-alanine

The method is limited in its application in two ways. The halogen acyl chloride for every amino acid does not exist, so that many amino acid radicles cannot be introduced. The chain of amino acids can be lengthened only from one end.

Method 3.

This method consists in using the acid chloride of the amino acid in place of the halogen acyl chloride. It is the most direct method and has the advantage over the previous method in that the amino acids can be united together at either end of their molecules, and by using the acid chloride of a dipeptide or polypeptide, the amino acids can be combined in every conceivable manner. The method could not be used at the beginning of Fischer's researches, as the acid chlorides of the amino acids were unknown and attempts to prepare them had failed. In 1904 Fischer discovered that the presence of a halogen acyl group in the molecule of an amino acid made it possible to prepare its acid chloride and that it was formed by the action of phosphorus pentachloride in the presence of acetyl chloride. It could be combined with the esters of amino acids or of polypeptides. Thus, bromisocapronyl-glycine chloride could be made into its acid chloride and combined with glycine ester to produce bromisocapronyl-glycine ester:

$$(C_4H_9).CHBr.CO—HN.CH_2.COCl + H_2N.CH_2.COOC_2H_5 \longrightarrow$$
$$(C_4H_9).CHBr.CO—HN.CH_2.CO—HN.CH_2.COOC_2H_5,$$

which on subsequent saponification and treatment with ammonia gave the tripeptide, leucyl-glycyl-glycine:

$$(C_4H_9).CH(NH_2).CO—HNCH_2.CO—HN.CH_2.COOH.$$

If bromisocapronyl-glycyl chloride were combined with glycyl-glycine ester, the tetrapeptide, leucyl-diglycyl-glycine was formed.

Not only was it possible to prepare the acid chloride of the amino acid by this method, but also the acid chloride of a di-, tri-, etc., peptide. Thus, the combination of bromisocapronyl-diglycyl-glycyl chloride with the esters of amino acids, dipeptides, etc., gave a pentapeptide, a hexapeptide, a heptapeptide; a decapeptide was produced, and finally a complex octadecapeptide with eighteen amino acid radicles.

The method is particularly useful for introducing the tyrosine and other complex radicles into a chain. It is employed most frequently in conjunction with the halogen acyl halide method as these compounds are more accessible.

Large numbers of dipeptides, tripeptides, tetrapeptides have been made by these methods. Every conceivable polypeptide could be made if the starting materials were available and the actual practical difficulties were not so great.

Properties of the polypeptides.

Most of the polypeptides are easily soluble in water, but many which contain the leucyl and phenylalanyl radicles are not soluble. They are not soluble in absolute alcohol, except leucyl-proline. They dissolve easily in dilute acids and alkalies forming salts, and give blue copper salts with copper hydroxide. The biuret reaction is given by the tetrapeptide of glycine, but other tripeptides show the reaction. The more complex polypeptides are precipitated by phosphotungstic acid, tannic acid and by concentrated solutions of ammonium sulphate. They resemble many of the natural proteins, differing only in the absence of tyrosine, tryptophan and other special radicles in their molecule. If they had been found in nature, they would undoubtedly have been classed as proteins. Another resemblance to natural proteins is their hydrolysis by trypsin, and erepsin. The hydrolysis by these enzymes depends largely upon the structure of the peptide (below).

Isomerism of the peptides.

It is possible to prepare by synthesis various isomeric compounds; in the case of dipeptides, two isomers exist, *e.g.*

glycyl-alanine and alanyl-glycine,

leucyl-tyrosine and tyrosyl-leucine.

There are six possible tripeptides containing the same three amino acids:

Glycyl-alanyl-leucine, Leucyl-glycyl-alanine,

Alanyl-glycyl-leucine, Alanyl-leucyl-glycine,

Glycyl-leucyl-alanine, Leucyl-alanyl-glycine.

Twelve isomers exist amongst the tetrapeptides, such as

Glycyl-tyrosyl-glycyl-alanine,

Glycyl-glycyl-tyrosyl-alanine,

Tyrosyl-glycyl-glycyl-alanine,

but all have not yet been made. In these isomers the amino acid should be regarded as present in its optically active form. If the amino acids be present in their racemic forms, the number of isomers is far greater.

It is partly owing to the immense number of isomers that the synthesis of a natural protein becomes almost an impossibility. Some guidance is

needed as to the order of the amino acids in the protein molecule. The isolation of some diketopiperazines from the products of hydrolysis of proteins and the action of trypsin affords some help, but much more information is wanted before a natural polypeptide can be made by synthesis.

The higher polypeptides, such as the octadecapeptide with 18 units in its molecule has a molecular weight of 1213. If such a compound contained tyrosine, phenylalanine, tryptophan and other radicles in place of most of the glycine units, its molecular weight would be from 3000 to 4000. Modern physical methods indicate molecular weights from 30,000 to 40,000. These proteins would contain from 200 to 250 amino acid radicles in their molecules. The synthesis of such a compound would be most difficult and is not likely to be attempted until the wonderful methods devised by Fischer have been further developed.

The action of enzymes on polypeptides.

One of the best proofs that proteins are built up like polypeptides is the hydrolysis of certain of the polypeptides by the action of enzymes. The action of trypsin upon a large number of the simple dipeptides was investigated by Fischer and Bergell, and by Fischer and Abderhalden. Some were hydrolysed, some were not hydrolysed. The following results may be taken as examples:

Hydrolysed	Not hydrolysed
alanyl-glycine	glycyl-alanine
alanyl-leucine	leucyl-alanine
glycyl-*l*-tyrosine	—
leucyl-*l*-tyrosine	—
d-alanyl-*d*-alanine	*d*-alanyl-*l*-alanine
d-alanyl-*l*-leucine	*l*-alanyl-*d*-alanine
d-alanyl-*l*-tyrosine	*l*-leucyl-glycine
l-leucyl-*l*-leucine	*l*-leucyl-*d*-leucine

It will first be noticed that only those dipeptides containing the natural optically active form of the amino acid are hydrolysed, and secondly that the order of combination affects the result, *e.g.* alanyl-glycine was hydrolysed, but not glycyl-alanine. There is unfortunately no regularity. In some cases with glycine at the end hydrolysis occurred, in other cases hydrolysis did not occur. The problem of the arrangement of the amino acids is also not helped by the more recent work of Waldschmidt-Leitz on the hydrolysis of peptides of various complexity. These researches distinguish the proteolytic enzymes as belonging to three groups:

Erepsin, which hydrolyses only the simple dipeptide, or tripeptide.

Trypsin, which hydrolyses the more complex peptone.

Trypsin-kinase (formerly trypsin) which hydrolyses the original protein and the peptone.

Pepsin was found by Fischer to have no action on any of the polypeptides. He was inclined to the view that the length of the polypeptide chain was not long enough for attack by pepsin, but did not exclude the view that other combinations of the amino acids might be present in the molecule and that

pepsin attacked these junctions with the liberation of several molecules of polypeptides.

The isolation of peptides and anhydrides from proteins.

A compound, termed leucinimide was isolated long ago from the products of hydrolysis of proteins by Bopp, and has been characterised as leucine anhydride. Fischer and Abderhalden in 1906 obtained the diketopiperazine of glycine and alanine from silk fibroin. This compound could arise from glycyl-alanine or from alanyl-glycine. The diketopiperazine of glycine and tyrosine was also isolated. It was shown that the former arose from glycyl-alanine and the latter from glycyl-tyrosine. Glycyl-leucine anhydride was isolated from edestin, and a tetrapeptide consisting of tyrosine, alanine and two molecules of glycine from silk fibroin. Many other anhydrides have been isolated from various proteins together with some dipeptides:

l-phenylalanyl-*d*-alanine anhydride from casein,

l-leucyl-*d*-valine anhydride from casein,

l-leucyl-*d*-glutamic acid from gliadin,

l-prolyl-*l*-phenylalanine from gliadin.

These compounds simply show that the two amino acids are probably joined together in the original molecule, but they do not show the order of combination, or whether the amino acids are at the end or in the middle of a chain. Their existence in the protein molecule is also open to doubt, as the anhydrides are easily produced by the action of the reagents used in their isolation. Every precaution was taken to avoid their secondary formation from amino acids, and it is most likely that anhydrides actually exist in the protein molecule.

The structure of the polypeptides.

The method of synthesis gives the structure of the polypeptides, as substituted acid amides. These compounds, like the amino acids, may exist in two forms:

$$H_2N.CHR.CO—NH.CHR.COOH \text{ and } H_2N.CHR.CO—HN.CHR.COO,$$

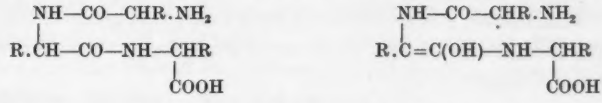
which may also have the lactim form:

$$H_2N.CHR.C(OH)=N.CHR.COOH \text{ and } H_2N.CHR.C(OH)=CHR.COO.$$

Indications that these isomeric forms of the polypeptides exist were noticed during their preparation. As it was impossible to decide the structure to adopt, the first formula is commonly used. The same kind of isomerism will be shown by the diketopiperazines; it is a possible structure, as will be shown later, in the protein molecule.

Hydrolysis of proteins after treatment with dilute alkali.

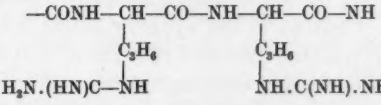
Proteins on solution in semi-normal alkali undergo a change in rotatory power. Hydrolysis does not occur as far as the formation of amino acids, but if the material be subsequently completely hydrolysed, some of the amino acids are obtained in the racemic form, whilst others are obtained in the usual optically active form. Dakin offered an explanation of this change suggesting that the amino acids in the chain underwent a keto-enol transformation:



It will be observed that the carbon atom at the end of the chain cannot undergo this transformation. Gelatin and a few other proteins have been examined in this way. Leucine, aspartic acid, phenylalanine, histidine and arginine were isolated in the racemic forms, whilst lysine, glutamic acid and a part of the alanine and proline were isolated in the optically active forms. Lysine and glutamic acid are thus probably at the end of a chain of amino acids. This explanation is not agreed to by Csonka and Horn.

The combination of the basic amino acids.

The basic amino acids, arginine, histidine and lysine, are capable of entering into combination with either their α-amino group, or with their special basic grouping, the ε-amino group in the case of lysine. Special investigations were made by Kossel to ascertain whether the guanidine group was free or combined. Kossel and Kennaway found that clupeine could be nitrated and changed into nitroclupeine. On hydrolysis of nitroclupeine they obtained nitro-arginine. The guanidine grouping was therefore not used in the linking of the amino acid, but remained free, thus



This was shown also for other proteins and is probably general in all proteins.

If proteins be treated with nitrous acid by the Van Slyke method, the amount of nitrogen evolved is exactly half that obtained for the amount of lysine produced in hydrolysis. This shows that only one of its two amino groups are in combination with another amino acid. The reaction of proteins with formaldehyde also corresponds with the amount of lysine with one free amino group.

After treating proteins with nitrous acid and subsequently hydrolysing them it has not been possible to isolate lysine.

There is thus much evidence that the ε-group of lysine is free. Histidine does not undergo any special reaction by means of which its iminazole group

can be detected. By analogy and from its structure, it is considered that the glyoxaline ring is not concerned in the linking of the amino acid.

The combination of the dibasic amino acids.

It is possible to effect combination of the dibasic amino acids, aspartic, glutamic and hydroxyglutamic, by either of their carboxyl groups. A dipeptide of one of these dibasic acids will exist in two isomeric forms. The comparison of the amount of ammonia evolved on hydrolysis with the amount of ammonia calculated, if in combination with a carboxyl group of these dibasic acids, shows in most cases a very close correspondence, but in other cases there is a marked difference:

	Ammonia calculated	Ammonia observed
Casein	1·4	1·6
Zein	2·3	3·6
Edestin	2·2	2·3
Phaseolin	2·4	2·1
Gliadin	4·4	5·1
Glutenin	2·8	4·0

As the proteins are almost neutral in reaction, this evidence is in favour of the second acid grouping of these compounds being combined with ammonia rather than with another amino acid. Physical methods also support the presence of amide groupings with the dibasic amino acids. By analogy it is assumed that the carboxyl group next to the amino group is used in the linking of these amino acids, but there is no direct evidence.

The additional carboxyl group of the dibasic amino acids, if not in combination with ammonia, will be capable of combination with other groupings of other amino acids, such as the hydroxyl groups of the hydroxy amino acids, or it may act in salt formation with the free amino groups of lysine or the guanidine group of arginine. Anhydride formation is a further possibility.

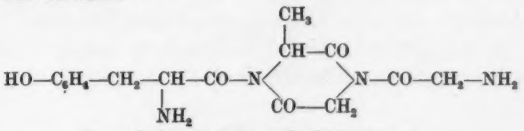
All amino acids appear to be in combination with their α-amino groups and the carboxyl group on the neighbouring carbon atom. It is not known whether the hydroxyl groups of the hydroxy amino acids are free or combined. The work of Rimington indicates that the hydroxyl group of hydroxyglutamic acid is in combination with phosphoric acid in casein, and possibly also with serine. There may therefore be ester combinations or ether combinations with the hydroxyl groups of these amino acids.

The presence of diketopiperazine rings in proteins.

Though the linking together of the amino acids as polypeptides is apparently the predominant form of union, the isolation of diketopiperazines from the products of hydrolysis under the most careful conditions of work so as to avoid their secondary formation must lead to the conclusion that such rings are present in the protein molecule. Their presence was not overlooked by Fischer, and is strongly advocated by Abderhalden.

Simple diketopiperazine rings composed of two amino acids have been

frequently isolated from the hydrolytic products of proteins, such as leucinimide, or leucine anhydride, and leucyl-valine anhydride. Abderhalden has specially studied the products from silk fibroin. In addition to the isolation of glycyl-tyrosine anhydride and glycyl-alanine anhydride he has indicated the presence of a more complex compound. The products of mild hydrolysis were reduced with sodium and alcohol so as to stabilise the ring compounds and substances which seemed to be the reduction products of glycyl-alanine and alanyl-serine anhydrides were obtained. Another substance appeared to be the reduction product of a complex containing two molecules of glycine, one of alanine and one of tyrosine. The original compound was also isolated in another experiment and its analysis as well as its products on hydrolysis agreed with the formula



Tyrosyl-alanyl-glycine-anhydride-glycine

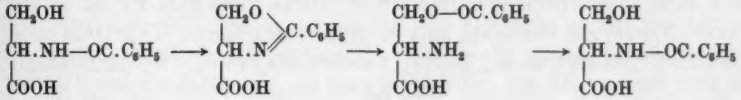
It closely resembled the tetrapeptide, glycyl-alanyl-glycyl-tyrosine, previously obtained by Fischer and Abderhalden.

Abderhalden has worked out a series of colour reactions for diketopiperazines which show their presence in the protein molecule. Some diketopiperazines yield oxamide on oxidation, which is obtainable by the direct oxidation of proteins.

There is thus considerable evidence for the presence of diketopiperazine rings in proteins.

Diketopiperazine rings with hydroxy-amino acids.

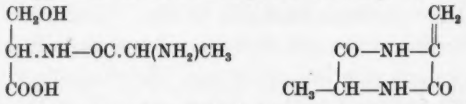
Dipeptides and diketopiperazines of serine have been studied by Bergmann and his colleagues, who have described some remarkable and important transformations in such compounds. Reference must first be made to the analogous compound, benzoyl serine. Benzoyl serine on treatment with thionyl chloride gave through loss of water and ring closure an oxazoline peptide. This compound was stable in neutral or alkaline solution, but in acid solution the ring was opened and the isomeric benzoyl ester peptide was formed in which the benzoyl group was attached to the OH group. Neutralisation of the solution then gave the original N-benzoyl serine. The changes were the following:



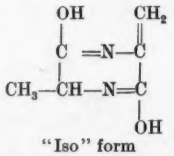
Benzoyl serine amide peptide — Oxazoline peptide — Ester peptide — Amide peptide

The reactions with alanyl-serine and with glycyl-serine were more complicated. The dipeptide of the former on treatment with thionyl chloride gave

a base containing chlorine, which formed an insoluble anhydride I, $C_6H_8O_2N_2$. This anhydride I appeared to be 3-methylene-6-methyl-2, 5-diketopiperazine:

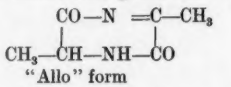


since on hydrolysis it gave pyruvoyl-alanine, and on reduction gave alanine anhydride. It may have been formed by loss of $2H_2O$ from alanyl-serine. On solution in alkali and reprecipitation with acid, it was changed into another anhydride (anhydride II), considered at first to be of higher molecular weight $(C_6H_8O_2N_2)_2$, but later, shown to be of simple molecular weight and consequently isomeric. The formula



"Iso" form

was suggested for this isomer. This "iso" form could pass into another "allo" form on heating with dilute ammonia, guanidine or arginine:

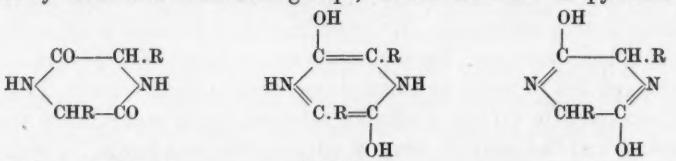


"Allo" form

The existence of compounds such as the above in proteins has not yet been proved. Their striking properties of lability and polymerisation offer explanation of many of the complex properties of the proteins, such as denaturation on heating, and the insolubility of the group of keratins.

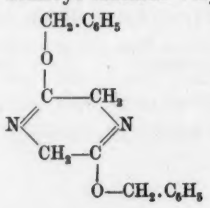
Isomeric forms of the diketopiperazines.

Like the dipeptides, the diketopiperazines may exist in two other modifications by enolisation of the keto-groups, the enol form and the pyrazine form:

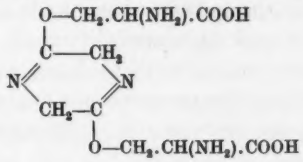


Ordinary diketo form | Enol form | Pyrazine form

Karrer and his collaborators have prepared substances of the pyrazine form, such as the benzoyl derivative of glycine anhydride by the action of benzyl chloride on the silver salt of glycine anhydride. This compound is hydrolysed into glycine and benzoyl chloride very rapidly by dilute acids:



If we had a compound such as the following, in which alanine is joined to glycine anhydride, hydrolysis would give serine and glycine:

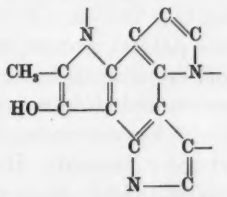


Serine might thus not actually be present in the protein molecule, though capable of being formed in the process of hydrolysis. Again, there is no evidence for this type of combination, but its possibility is of the greatest interest and must not be overlooked.

Besides the above, Karrer has suggested several other possible rings formed by enolisation of dipeptides and then followed by ring closure. Any of these structures might be present. Their preparation and examination is awaited.

Pyrrole rings.

Ring structures in the form of condensed pyrrole rings have been advocated by Troensegaard. The evidence for such structures is scanty, but they would explain the formation of pyrrole compounds on dry distillation of proteins and so connect proteins with chlorophyll and haematin. The formation of amino acids from such a structure, though possible, is not in accordance with the mechanism of hydrolysis by enzymes, which require acid amide linkings. This ring structure is considered by most authors as very unlikely.



It seems that every conceivable structure has been put forward to explain the formula of proteins. Except the last, all are based on the acid amide structure or peptide linking. Trypsin and erepsin attack this linking if it be in simple chain form, but they do not hydrolyse the closed ring form of the diketopiperazine. Pepsin attacks neither the simple chain linking, nor the diketopiperazine ring. The explanation of the action of pepsin has not yet been solved. Fischer's supposition that the chain of amino acids of the synthetical polypeptides was not long enough has not been tested with the higher polypeptides and requires the test of experiment. Another form of linking between the amino acids is difficult to imagine, if we exclude ester and ether linkings. Long chains of amino acids joined by diketopiperazine rings at the ends so as to give a more compact molecule is very plausible.

Pepsin might break the junction of the diketopiperazine rings and thus give several large molecules of chain polypeptides. Another possibility is the hydrolysis of polypeptide chains with dibasic acids at the ends in which the second carboxyl group is used; this possibility needs the test of experiment. The compact form of structure indicated by X-ray examination which shows repeated units of simple peptides necessitates a linking to repeat the peptide unit. The chemistry of the proteins still offers many openings for further research.

Part IV. Proteins in nutrition.

Proteins are hydrolysed by boiling with acids into a mixture of 19 or 20 amino acids. Amino acids are also produced by the action of trypsin and other enzymes on proteins. Though pepsin does not hydrolyse proteins further than a mixture of proteoses and peptone, yet its action has a distinct effect upon the completeness of the action of trypsin. Fischer and Abderhalden found that proline and phenyl-alanine were not separated from casein by trypsin unless the casein had been previously digested with pepsin. The two enzymes acting one after another convert 75 per cent. or more of the protein into amino acids. In normal digestion of food the more complex products are finally acted upon by erepsin, and the proteins are completely converted into amino acids. It would thus appear that the amino acids are the actual substances assimilated in protein nutrition.

On account of the difficulty of preparing each of the amino acids in sufficient quantity so as to prepare mixtures corresponding closely with the mixture obtainable from casein or other proteins, the direct test that amino acids are assimilated and can take the place of protein in the food is almost impossible to carry out, but a natural mixture, prepared by prolonged digestion with pepsin, trypsin and erepsin not showing the presence of any more complex product can be made. Abderhalden prepared such a mixture from meat, and in combination with carbohydrate, fat and mineral salts in the proper proportions administered it to a dog. During the course of 100 days the dog gained nearly 10 kg. in weight and appeared perfectly normal and able to continue on the diet. This experiment with many others was one of the first proofs that the amino acids were the essential parts of protein in nutrition. Physiologists have subsequently shown clearly that the amino acids are absorbed from the intestine into the blood and circulate in the blood to the various tissues which can store them and assimilate them as required. Digestion and assimilation are therefore a breaking up of food protein and rebuilding of body protein from the amino acids. The different proteins have different compositions as shown in Table II. If the body protein is very different from the food protein then some of the amino acids may be in excess and others in default. The process is like pulling down a house and rebuilding it with the same materials, but if the new shape is not exactly the same, the parts will not be in the right proportions; at the same time there will be a

certain loss of material through breakage and in the case of the protein synthesis in the body there is such loss by oxidation of the amino acids.

The chemical analysis of proteins shows that they are not the same and that they should not be expected to have the same value in nutrition. The usual practice of judging the protein value of a foodstuff from the amount of nitrogen is quite insufficient. This figure simply indicates the total quantity of protein. A protein ought to be valued by the amount of each amino acid, *i.e.* by its tryptophan, arginine, or leucine, etc., value. This valuation is not usually considered, partly because our information of the amino acids is not yet sufficient, partly because some amino acids may be more necessary than others and partly because so many have to be considered. A great deal of work has been done upon the values of the different amino acids. The results are of interest and of importance.

The values of individual amino acids.

The direct method of ascertaining the values of the several amino acids in nutrition by feeding mixtures of the pure amino acids is so difficult to carry out, as sufficient supplies of the various compounds are not easy to prepare, that it has not been performed till quite recently. The problem is more easily investigated by feeding animals with an incomplete protein and supplementing it with the missing amino acid or amino acids, or the investigation can be made by using the products of hydrolysis of a complete protein such as casein and removing from the mixture a single amino acid, or a group of amino acids. The values of several amino acids have been tested in this way.

(1) *Tryptophan.*

One of the earliest experiments to examine individual amino acids was with tryptophan. It was made by Wilcock and Hopkins with the incomplete protein zein. This protein was given in an otherwise suitable diet to two sets of young mice, the one set without any additional tryptophan, the other with 2 per cent. of the amount of protein consisting of tryptophan. Those without tryptophan lost weight and died in 16 days, those with tryptophan also declined in weight but survived till the 30th day. Adult mice lived 27 days without tryptophan, 49 days with tryptophan. Tryptophan had thus an appreciable effect on the length of life of the animals. The failure of the animals was probably due to zein being incomplete in respect of other amino acids. The value of tryptophan was tested more thoroughly by Ackroyd and Hopkins, who used a mixture of amino acids prepared from casein by acid hydrolysis, which contains all the amino acids except tryptophan which is destroyed in the hydrolysis. Animals grew quite well on the hydrolysed casein supplemented with tryptophan for 13 days; the tryptophan was then omitted and a marked decline in weight followed. On the 35th day the tryptophan was again added to the food and growth was resumed. Tryptophan was thus shown to be an essential amino acid in nutrition and it cannot be

synthesised in the animal body from other amino acids, or from other parts of the food. The essential need of tryptophan has been confirmed by Berg, Rose and Marvel, who found that it was better to give it half at a time rather than in one dose.

(2) *Lysine.*

Osborne and Mendel made experiments with the disproportionate protein, wheat gliadin, which contains tryptophan but very little lysine. Adult rats were maintained for periods as long as 500 days on this protein, but young rats maintained for long periods failed to grow; they increased in size on adding lysine to the diet. In a later series of experiments with wheat gliadin, the addition of lysine was made at intervals. During the days when lysine was added there was growth, but not when it was absent. The growth curve of the animals appeared as a series of steps. Lysine was regarded by these authors as essential for growth. The growth effect of lysine was again shown by Buckner, Nollau and Kastle in some experiments with chickens at a poultry farm. Some were given a protein mixture of high lysine content, and others of a low lysine content. Those with the higher lysine content showed more rapid growth. There may have been other factors in the diet which contributed to the better growth so that the experiment, though so striking, cannot be regarded as accurate.

Osborne and Mendel have pointed out that every amino acid has a limiting effect upon growth. It is not correct to look upon any single amino acid as being especially necessary for growth. To secure maintenance each amino acid must exist in the food in a minimal quantity; growth cannot take place till this minimum is exceeded.

(3) *Histidine and arginine.*

As these two amino acids can be removed completely from the hydrolysis products of proteins by means of silver sulphate and baryta, and also separated from one another, the opportunity is given of testing whether their presence is essential. Ackroyd and Hopkins showed that the removal of both compounds from the mixture caused loss of weight. If either were then added to the mixture, then the loss of weight of the animals was distinctly lessened, and some growth was possible. It was therefore believed that these two amino acids were interchangeable. Support for this interrelationship was given by experiments by Geiling, but such interrelationship of histidine and arginine has not been confirmed. Neither Stewart, nor Rose with his co-workers, could secure this result. Harrow and Sherwin found that histidine was essential, but that arginine was not necessary. The difference in results seems to be due to the incomplete separation of histidine and arginine by the silver and baryta method which is difficult to effect and the consequence is that the arginine fraction contains some histidine. Bunney and Rose have repeated the experiments, using flavianic acid to remove arginine. The animals

grew well in the absence of arginine. Histidine is an essential amino acid, but not arginine.

(4) *Cystine and methionine.*

The presence of sulphur in cystine and methionine would lead to the *a priori* conclusion that one or both of these compounds would be necessary as a dietary property of proteins.

(*a*) *Cystine.* The need of the presence of cystine amongst the amino acids has been tried by feeding rats on proteins with very little or possibly no cystine. Such proteins are those of the legumes. It was shown by Johns and Finks that there was slow growth with the proteins of the navy bean, lentils and other legumes, but normal growth if they were supplemented with 2 per cent. of its amount of cystine.

Beadles, Braman and Mitchell have found that the proteins of peas and of potatoes are lacking in cystine. Animals showed better growth if the diet contained an addition of 0·24 per cent. of cystine.

Osborne and Mendel proved the importance of cystine in dietary experiments with casein, which contains very small amounts of cystine. Rats on a diet containing 15 per cent. casein showed normal growth, but less growth on the same diet with 9 per cent. On this diet the growth became normal on supplementing the casein with cystine.

Cystine is most abundant in the keratins, hair and wool. Examination of the hair of animals on diets poor in cystine and on diets with an addition of cystine showed differences. The hair not only showed better growth but was more abundant with the addition of cystine. According to Lightbody and Lewis, the demand for cystine for the growth of hair is secondary to the demand for the growth of the whole animal.

(*b*) *Methionine.* The presence of methionine is known only in a few proteins. Owing to the difficulty of its separation from a mixture of amino acids, its value has not yet been tested.

(5) *Phenylalanine and tyrosine.*

These amino acids containing aromatic nuclei would be expected to be essential in nutrition. Some proteins lack tyrosine, but all contain phenylalanine. Tyrosine is very insoluble and is consequently capable of removal from the products of hydrolysis, but it is very difficult to remove phenylalanine. Abderhalden tested a mixture of amino acids from which he had separated the tyrosine as completely as possible and concluded that tyrosine could not be dispensed with. The experiment was made before the importance of vitamins in nutrition was recognised. Totani, on repeating the experiment with a diet containing vitamins, concluded that tyrosine was not essential, as the growth of rats with and without an addition of tyrosine was almost the same. In experiments with a mixture from which tyrosine is removed

by crystallisation it has frequently if not always been noted that the solution gives a Millon reaction, which indicates that some tyrosine still remains.

The two aromatic amino acids appear to be closely connected in metabolism. Both give rise to homogentisic acid in cases of alkaptonuria. Phenylalanine may therefore be oxidisable to tyrosine in the animal body. The amount of phenylalanine in the ordinary edible proteins is probably always sufficient to supply all the aromatic nuclei which are needed.

(6) *Proline and hydroxyproline.*

These two amino acids are almost universally present in proteins. Though proline can be fairly easily removed from a mixture, the complete separation of hydroxyproline is very difficult. Satisfactory experiments on their nutritive values are almost impossible to perform.

Barnett Sure made use of the protein, arachin, from pea nuts which contains very little proline. Owing to the peculiar indigestibility of this protein, no conclusion could be arrived at. On trying experiments with lactalbumin and with edestin which contain very little proline Sure believed that proline was essential. Edestin supplemented with cystine and gelatin gave good growth. With a diet containing 9 per cent. of edestin, the addition of cystine, lysine and proline had no effect, but with a diet containing 6 per cent. of edestin, the addition of these amino acids had a marked effect.

The close chemical relationship of proline and hydroxyproline with glutamic and hydroxyglutamic acids must be considered before a final decision of the need of these compounds is made.

(7) *Aspartic, glutamic and hydroxyglutamic acids.*

Nothing definite is known about the values of these compounds. All proteins contain them in greater or smaller quantities. There is no satisfactory way of completely removing them from a mixture. Bunney and Rose have tried a diet free from arginine and containing smaller amounts of these dibasic acids than is present in the products of hydrolysis of casein. The animals were able to grow normally.

Hydroxyglutamic acid is absent from edestin. Osborne, Leavenworth and Nolan consider it is not essential, as animals grow with edestin as source of protein.

Chemically, glutamic acid and hydroxyglutamic acid are easily changed into their anhydrides, the corresponding pyrrolidone carboxylic acids. These acids on reduction give proline and hydroxyproline. If the animal body is able to change these amino acids into their anhydrides and then reduce the pyrrolidone carboxylic acids, there will be interrelationship of these four compounds.

It is also possible that hydroxyglutamic acid is formed by oxidation in the body from glutamic acid in a similar way to that of tyrosine formation from phenylalanine.

(8) *The simple mono-amino acids.*

It is very difficult to separate a single mono amino acid from the mixture of hydrolysis products and leave the others. All proteins contain several members of this group. The essential nature of any of these compounds in nutrition has to be judged indirectly. There are several important observations for consideration.

(*a*) *Glycine.* There are many reasons for believing that glycine is not an essential compound in proteins and that it can be synthesised by animals. It is not contained in casein nor in lactalbumin, and all young animals grow on this mixture of proteins in the milk. Benzoic acid, if administered to animals, is excreted in the form of hippuric acid or benzoyl-glycine. This detoxicating action of glycine is continuously effected on diets of casein without loss of body weight by the animals. Glycine is produced from some other compound in the animal body, but its method of synthesis by animals is not known.

(*b*) *Alanine.* Alanine has been found in all proteins. It may almost definitely be regarded as non-essential. It has been conclusively shown that it can be produced by the liver from the ammonium salt of pyruvic acid, and also from ammonium lactate:

$$\begin{array}{l} CH_3 \\ | \\ CO \\ | \\ COOH \end{array} + NH_3 + H_2 = \begin{array}{l} CH_3 \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} + H_2O$$

Alanine was isolated after introduction of the ammonium salts of these acids into blood passing through the liver (Embden and Schmitz).

Pyruvic acid is produced from glucose in alcoholic fermentation as a stage in the formation of alcohol and carbon dioxide, and it is also a stage in the formation of lactic acid in fermentation. Lactic acid and pyruvic acid are related in the metabolism of muscle and arise from carbohydrate. Alanine is therefore probably formed by synthesis from carbohydrate not only in the liver, but also in muscle and other organs.

(*c*) *Serine.* The value of serine is quite unknown. Its presence is only known definitely in a few proteins on account of the difficulty of its isolation. It is possible that it may be formed from alanine by oxidation in the same way as hydroxyglutamic acid might be produced from glutamic acid.

(*d*) *Valine, leucine and isoleucine.* There is no information about the function of these three mono-amino acids. The fact that they contain branched chains of carbon atoms would lead to a belief that they are necessary constituents of edible proteins.

(9) *A new amino acid.*

The direct experiment of feeding animals upon a mixture of pure amino acids has been tried by Rose, who has accumulated a sufficient stock of the twenty amino acids found in proteins. Some were prepared by synthesis

and some directly from proteins. The synthetical amino acids were given in double the quantity of that which is present in the proteins, as the unnatural amino acid is not assimilated by animals. On mixing all the amino acids in suitable proportions resembling the mixture from hydrolysed casein as substitute for the protein of the food and supplying all the necessary vitamins, carbohydrate, fat and mineral salts, Rose found that rats did not grow. The mixture was first supplemented with casein or gelatin or other protein. With the supplement the rats grew normally. The mixture of products from hydrolysed casein was separated by the method of Dakin with butyl alcohol and each fraction tested as a supplement to the mixture. The effective fraction was that of the mono-amino acids in the butyl alcohol extract, but not the proline fraction. Since all the known amino acids contained in this fraction were in the mixture, the conclusion is that some other unknown amino acid is present in that fraction. This fraction of amino acids has been found the most difficult to separate and generally neglected. Animal experimentation will now give a stimulus to further chemical analysis of proteins.

The presence of another amino acid which is essential will help to explain the impossibility of making gelatin with supplements of tyrosine, tryptophan, cystine and histidine a satisfactory protein in nutrition. Jackson, Sommer and Rose tested the supplementation of gelatin with the above four compounds together with extra phenylalanine, aspartic acid, glutamic acid, valine, isoleucine and norleucine and still found that growth of rats was not normal. Supplementing with casein or gliadin produced normal growth.

The results of feeding experiments have thus established that tryptophan, lysine, histidine and cystine are indispensable; that glycine and alanine can be formed in the animal body; that phenylalanine and tyrosine are interrelated; that arginine and hydroxyglutamic acid are not necessary. The essential nature of the other amino acids is not known.

The nutritive values of proteins.

The amino acid composition of proteins applies directly to the nutritive values of the proteins themselves. The early experiments of Osborne and Mendel upon the different values of pure proteins prepared from various sources cannot now be considered reliable, as they were carried out before vitamins were discovered and the different growth effects attributed to the proteins may really have been due to shortage of vitamins. In fact these experiments led largely to the discovery of the two groups of vitamins, the fat-soluble and the water-soluble. A great many other experiments on the comparative values of proteins need repetition on this basis with the full recognition of vitamin requirements.

Proteins of animal origin.

Lactalbumin and casein were compared by Osborne and Mendel by feeding varying quantities in an otherwise complete diet, the object being to ascertain the minimum amount which would give normal or nearly normal growth of rats. Thus 2 per cent. lactalbumin gave maintenance, 4·5 per cent. gave growth. Slight growth was observed with 4·5 per cent. of edestin. There was no growth with 4·5 per cent. casein unless it was supplemented with cystine. Lactalbumin appeared as a better protein than casein. The superior value of lactalbumin to casein has never been confirmed. In all subsequent trials casein has been found definitely better for growth than lactalbumin.

Some experiments (not yet published) by the author also showed the superiority of casein over lactalbumin, and over meat proteins and fish proteins. The latter two proteins were about equal; the lowest value was shown by blood proteins.

Proteins of legumes and nuts.

Though the pulses contain comparatively large amounts of protein equal in quantity to that in animal flesh, the quality of the protein is distinctly inferior. The proteins of legumes in general contain insufficient cystine, but this deficiency is not the only explanation of the inferiority. Lack of digestibility is partly the reason. The proteins of legumes are better assimilated after cooking than in the raw state. Soya bean protein appears to be the best. The proteins of the pea nut are peculiar in digestibility. Further experiments on the inadequacy of the legume proteins are needed. The amino acid content of the pure proteins except for the absence of cystine resembles that of animal proteins. The pure protein is not so poor in nutritive value as the unisolated protein in the seed, perhaps because of a difference in digestibility. Nut proteins have a high proportion of the di-amino acids. Normal growth of animals has been observed with coconut press cake, walnut and other nuts.

Proteins of cereals.

The chief proteins of cereals belong to the groups of gliadins and glutelins. The inadequacy of zein and the small amount of lysine in wheat gliadin and other gliadins as well as other disproportionate quantities of amino acids show that these proteins are inferior. The glutelins are more satisfactory for growth. The lack in the one group is largely compensated by the supply in the other. The whole grain also contains small amounts of albumin and other proteins. The protein of the germ, mainly albumin, resembles the animal albumins. The whole grain will contain a mixture of proteins with an amino acid content approaching that of an animal protein. Growth has been observed on the whole grain if suitably mixed with mineral salts and vitamins. The inferiority of the mixed proteins of cereals as promoters of growth is not so much due to lack of quality as to the small quantity which is present. The protein ratio

of the components of grains is about 1 to 10; for normal growth the protein needs to be raised to 1 to 5. Supplementing the grain with gluten (the mixture of gliadin and glutelin) has not produced normal growth, but good growth has followed supplementing with wheat germ which contains the albumin. The author has observed growth with an addition of wheat germ to wheat almost as good as with an addition of fishmeal or meatmeal.

The division of proteins into good and bad in nutrition needs consideration from several aspects. Though the individual proteins of grains are of poor quality, their mixture is relatively good and better if mixed with wheat germ. The total quantity is too small. In legumes there is an ample quantity of protein, but its digestion is difficult and the protein is deficient in cystine and possibly in other amino acids. The proteins of animal origin are better; the best protein seems to be casein; meat and fish proteins are very similar.

A great deal of work has been carried out on the values of proteins in nutrition both in animals and man. A fuller description of these experiments is outside the scope of this monograph. A review is given by McCollum and Simmonds in *The Newer Knowledge of Nutrition.*

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36. CHEESE-RIPENING STUDIES[1]

NITROGEN DISTRIBUTION IN KINGSTON CHEESE-RIPENING

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(With 3 Figures.)

The process employed in the manufacture of Kingston cheese was evolved and reported upon in 1911 (1). By certain modifications of the principles that govern the making of "hard-pressed" cheese, one that is mature and ready for consumption within 14 days after making is obtained. The typical Kingston cheese is 1 lb. in weight, the flavour is mild and the texture soft, as compared with that of Cheddar cheese, and less granular than that of the Cheshire variety. Such a cheese is clearly suitable as a medium for study in cheese-ripening, and for several years, intermittently, a study of the bacteriology of the cheese has been in progress (2, 3). Since the earlier work was recorded (1, 3) the employment of pasteurised milk instead of raw untreated milk has become the routine practice in our cheese-making; and as from June, 1924, all milk used for this purpose has been pasteurised for 30 min. at 142° F. (2)

The founding of the Empire Marketing Board-University of British Columbia Joint Research Fund enabled us to engage in a biochemical and bacteriological investigation of the course of ripening in Kingston cheese. Methods employed by workers in various fields of study have been used for the determination of the nitrogen distribution. The different nitrogen fractions which were determined are described below, the fractions being expressed as percentages of the total nitrogen.

Soluble nitrogen is the nitrogen of the filtered aqueous solution of the cheese or, in other words, is the nitrogen of the soluble protein *plus* that of the protein decomposition products present in the cheese.

The *amide nitrogen* is the nitrogen of substances not precipitated by phosphotungstic acid, under definite conditions of acidity, from the soluble nitrogen solution.

For the determination of soluble nitrogen the technique followed in strict detail is that given by Barthel (4).

Orla-Jensen's methods. It will be seen that we have also applied to the work on cheese the methods used by Orla-Jensen (5, 6) for the determinations of soluble nitrogen and amino nitrogen (decomposition nitrogen) in chalk milk cultures. The soluble nitrogen thus determined is designated throughout as

[1] These studies are provided for by a Research Fund established jointly by the Empire Marketing Board and the University of British Columbia.

soluble nitrogen (*O.-J.*), and *amino nitrogen* refers to that nitrogen fraction determined according to the method described and used by Orla-Jensen for the determination of DN (decomposition nitrogen) in chalk milk cultures.

In addition to carrying out determinations on cheese at various stages of ripening according to the methods already cited, it has been thought desirable to determine whether or not the method developed by Wasteneys and Borsook (7) for the fractional analysis of incomplete protein hydrolysates could be applied to the study of the protein breakdown during cheese-ripening. This method defines the nature and amount of the specific protein decomposition fractions (proteose, peptone and subpeptone) resulting from the breakdown of the cheese protein. It was considered possible that this method would secure more definite information regarding the nature of the proteolytic enzymes elaborated by the bacteria found to be prevalent in the cheese.

The method of Wasteneys and Borsook for the fractional analysis of incomplete protein hydrolysates has stood the test of continued use by different workers for a number of years. In following the stages of the breakdown of protein by the action of defined enzymes, proteose was found to be the principal product in a peptic hydrolysis of protein (10), the proteose representing in some cases 60–70 per cent. of the total hydrolysis, the peptone making up about 30 per cent., and the subpeptone constituting the smallest fraction. An entirely different picture is presented by a tryptic (pancreatic extract[1]) hydrolysate (11). In this hydrolysate there is very little or no proteose, 20–30 per cent. peptone, by far the largest fraction being the sub-peptone.

Employing the methods described above, a comprehensive study of the protein breakdown during the ripening of Kingston cheese was undertaken.

Methods. Determinations were carried out on cheeses of the same "make" from the day of making to the 98th day of ripening at intervals defined and recorded in the tables and figures.

Sampling. One entire cheese was used for each complete examination. After removing the thin rind, the cheese was ground in a small grating mill. The ground cheese was thoroughly mixed and determinations for total nitrogen were done in triplicate on a weight of approximately 1 g.

Analytical methods. For the determination of the nitrogen distribution, two 25 g. portions of cheese were stirred with 100 c.c. of water at 50° C. and transferred to two 250 c.c. Stohmann flasks. The volume was brought up to about 200 c.c. with water, 8 drops of 40 per cent. formalin were added and the flasks shaken for a period of 24 hours in a mechanical shaker. Each flask was then made up to the mark and filtered. The filtrates were combined (filtrate 1). Two 25 c.c. portions of filtrate 1 were taken for the determination of *soluble nitrogen*.

To 50 c.c. of filtrate 1, 30 c.c. of 25 per cent. sulphuric acid (1 volume concentrated sulphuric acid, 4 volumes water), 10 c.c. of water and 10 c.c.

[1] A pancreatic extract (12) contains not one enzyme only, but at least three different proteolytic enzymes—trypsin (non-activated), trypsin-kinase and erepsin.

of 19 per cent. phosphotungstic acid were added. After standing 24 hours the precipitate was filtered off and two 25 c.c. portions of the filtrate were taken for the determination of *amide nitrogen* (phosphotungstic filtrate).

To another 50 c.c. of filtrate 1 were added 15 c.c. 1 per cent. acetic acid and 100 c.c. water. The solution was heated in a boiling water bath for 30 min., cooled, made up to a volume of 250 c.c. and filtered. Two 50 c.c. portions of this filtrate (filtrate 2) were taken for the determination of *soluble nitrogen* (*O.-J.*) (acetic acid filtrate).

To 100 c.c. of filtrate 2 were added 30 c.c. of 25 per cent. sulphuric acid, 10 c.c. water and 10 c.c. of 19 per cent. phosphotungstic acid. After standing 24 hours the precipitate was filtered off, and two 50 c.c. portions of the filtrate were taken for the determination of *amino nitrogen* (phosphotungstic filtrate).

In the application of the technique of Wasteneys and Borsook to the analysis of cheese, 150 c.c. of filtrate 1 were taken: to it were added 30 c.c. of 20 per cent. trichloracetic acid and the mixture was allowed to stand for 1 hour. The solution was filtered (filtrate 3). The filtrate was divided into two 70 c.c. portions (filtrate 3 A and filtrate 3 B), which were then placed in a boiling water bath for 3 hours to decompose the trichloracetic acid, and to drive off the resulting carbon dioxide and chloroform. Each flask was now cooled to room temperature, was filtered and made up to the original 70 c.c. volume. Two 5 c.c. portions of each filtrate, filtrate 3 A and filtrate 3 B, were taken for the determination of *soluble nitrogen* (*W. and B.*): (trichloracetic acid filtrate), so that results in duplicate of duplicate portions are available.

To the remainder of filtrate 3 A and 3 B respectively (approximately 60 c.c.) 22 g. of anhydrous sodium sulphate were added. The mixtures of salt (Na_2SO_4) and solution were kept at 35° C. for 1 hour. The solutions were then placed in a water-jacketed filter, maintained at 33° C. and allowed to filter up to the mark into 25 c.c. volumetric flasks without cooling (filtrate 4 A and filtrate 4 B); these were at once washed quantitatively into 50 c.c. volumetric flasks and made up to the mark. Two 10 c.c. portions of filtrates 4 A and 4 B respectively were taken for nitrogen estimation. The difference between the results of these nitrogen determinations—allowing for the dilution—and the nitrogen results of filtrates 3 A and 3 B respectively is the *proteose nitrogen* (sodium sulphate precipitate).

25 c.c. of each of the filtrates 4 A and 4 B were pipetted into 200 c.c. Erlenmeyer flasks; to each were added 25 c.c. of 2·21 normal sodium hydroxide, and 125 c.c. of 20 per cent. tannic acid dissolved in 0·1 normal sulphuric acid containing 20 per cent. sodium sulphate. After thorough shaking, the mixtures were kept at 18–20° C. for 3 hours and then filtered. Two 30 c.c. portions of each of these tannic acid filtrates were then taken for nitrogen determination—*the sub-peptone nitrogen*. The difference between the results of these total nitrogen determinations and the total nitrogen determinations on filtrates 4 A and 4 B respectively is *peptone nitrogen* (tannic acid precipitate).

In calculating the results from the analytical data, due account is to be taken of volume changes. In the determination of proteose nitrogen by the method of Wasteneys and Borsook a volume change which must not be overlooked is the volume increase due not to dilution but to the heating of the solution to 33° C. The allowance for the increase of volume due to heating to 33° C. is made by noting the increase in volume of water at 20° C. when saturated with sodium sulphate at 33° C. The determination of this volume allowance is carried out in a 25 c.c. volumetric flask with graduated neck. An example of the order of magnitude of the increase is that 22·5 c.c. of water at 20° C. when saturated with sodium sulphate at 33° C., occupied 25 c.c. The factor for volume increase due to heating is therefore 25/22·5.

The results thus obtained are expressed in percentage of the total nitrogen of the particular cheese upon which the determination was carried out. As calculated in this manner, the results are shown in Table I and Fig. 1.

Discussion on data shown in tables and figures.

Total nitrogen.

The total nitrogen figures in general show a steady rise as the ripening progresses. This rise in the total nitrogen figure is attributed to the loss of moisture from the cheese as the period of holding in the ripening room is extended. From Table I and Fig. 1 it is seen that on the twelfth and thirteenth days of ripening the total nitrogen contents of these cheese are widely divergent. The total nitrogens and also the results of other nitrogen fractions would indicate that these two cheese are not representative of normal ripening and hence that the data from these cheese are not typical of the sequential changes occurring during the ripening process[1].

Soluble nitrogen.

On the day of making, just after the cheese has been taken from the press, the soluble nitrogen is 12·35 per cent. of the total nitrogen. As is to be seen in Table I and Fig. 1, the soluble nitrogen content rises steadily until on the 98th day it constituted 33·19 per cent. of the total nitrogen.

Soluble nitrogen (*O.-J.*) *and soluble nitrogen* (*W. and B.*).

Soluble nitrogen (*O.-J.*) represents the nitrogen content of the soluble nitrogen solution after it has been boiled with very dilute acetic acid.

Soluble nitrogen (*W. and B.*) stands for the nitrogen content of the soluble nitrogen solution after precipitation with trichloracetic acid. By the latter

[1] For a year and a half the practice has been to ripen Kingston cheese at 64° F. in a room controlled by thermostats. After pressing, a procedure lasting for 3 hours, the cheese would be retained in the press-room for almost 2 days, whereupon they would be removed to the control-ripening room. At the time the determinations reported upon in this paper were made, the amendment to the usual practice had not been adopted. We suggest that the idiosyncrasy with respect to the nitrogen content and therefore the moisture content of these two cheese may have been due to the variation in temperature during the 2 days prior to being removed to the control-ripening room.

procedure all protein in the solution is removed, whereas in the former procedure only that protein coagulable by heat and acetic acid is removed. On the whole the soluble nitrogen (O.-J.) and soluble nitrogen (W. and B.) results run parallel with, but at a lower level than, those of the soluble nitrogen. The soluble nitrogen (W. and B.) is in every case lower than the soluble nitrogen (O.-J.), indicating the more complete removal of protein by the trichloracetic acid precipitation.

Table I. *Nitrogen distribution in Kingston cheese-ripening.*

Results are expressed in percentage of the total nitrogen of the respective cheese of the same "make" at the age here defined*.

Age of cheese in days	pH	Total nitrogen (%)	Soluble nitrogen (B.) (water-soluble)	Soluble nitrogen (O.-J.) (acetic acid-soluble)	Soluble nitrogen (W. and B.) (trichloracetic acid-soluble)	"Amide" nitrogen (phosphotungstic acid filtrate)	"Amino" nitrogen (phosphotungstic acid filtrate)	Soluble protein nitrogen (trichloracetic acid precipitate)	Proteose nitrogen (sodium sulphate precipitate)	Peptone nitrogen (tannic acid precipitate)	Sub-peptone nitrogen (tannic acid filtrate)
Day of making	.	2·704	12·35	7·13	4·88	0·947	4·10	7·47	2·15	2·73	0·00
1	5·50	2·779	13·60	8·70	5·94	1·756	4·21	7·66	2·13	2·70	1·11
2	5·40	2·930	18·19	10·34	8·96	2·24	5·53	11·23	1·98	3·31	1·67
4	5·35	2·906	15·52	11·26	7·43	2·202	6·19	8·09	1·69	3·61	2·13
5	5·35	2·886	16·63	10·80	8·31	2·245	5·20	8·32	2·04	3·50	2·77
6	.	3·095	17·20	9·58	8·46	2·83	5·74	8·74	2·13	3·52	2·81
7	5·35	2·895	17·92	10·46	9·60	2·653	4·98	8·32	2·80	3·80	3·00
8	.	3·000	18·63	10·34	9·33	2·507	6·50	9·30	2·23	4·00	3·10
9	.	3·106	17·53	12·62	9·72	3·193	7·80	7·81	2·35	4·18	3·19
11	5·30	3·029	17·98	13·92	12·67	3·592	8·88	5·31	4·05	4·96	3·66
12	.	2·660	18·94	14·60	12·60	3·331	.	6·34	3·02	5·42	4·16
13	.	3·498	17·56	12·44	12·01	3·152	8·44	5·55	3·59	4·54	3·88
21	5·10	3·283	22·54	16·44	14·62	3·777	9·14	7·92	4·08	5·46	5·08
28	.	3·300	23·03	19·34	17·15	4·54	13·54	5·88	4·42	7·67	5·06
35	5·10	3·260	.	21·60	19·14	5·18	13·16	.	4·54	9·20	5·40
42	5·05	3·380	26·27	23·60	20·00	6·57	13·84	6·27	3·64	11·06	5·30
49	5·10	3·420	26·73	24·04	19·89	5·62	16·14	6·84	4·04	10·62	5·23
63	.	3·534	32·71	28·21	22·89	7·24	22·07	9·82	3·40	11·42	8·07
84	5·30	3·553	33·27	30·09	26·73	9·08	24·23	6·54	3·09	15·62	8·02
98	.	3·498	33·19	30·36	27·24	8·95	24·87	5·95	2·12	17·46	7·64

* As is to be seen in (1), (2) the cheese is pressed for 3 hours only: the total nitrogen determinations on the cheese of the day of making were made immediately the cheese came from the press.

It can be taken that the soluble nitrogen (W. and B.) does not include any protein nitrogen but that it is made up entirely of protein decomposition products (7) or, interpreted in terms of the present study, that the soluble nitrogen (W. and B.) is made up of non-protein-nitrogenous compounds resulting from the action of proteolytic enzymes upon the cheese protein during the cheese-ripening process.

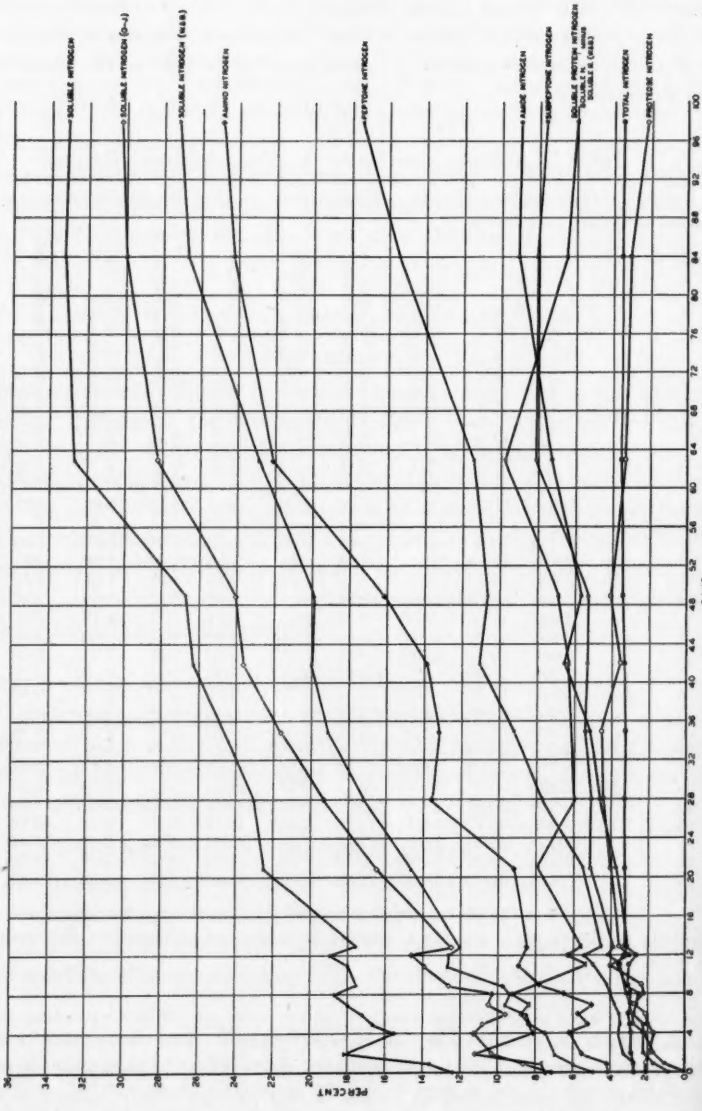

FIGURE I—NITROGEN DISTRIBUTION IN KINGSTON CHEESE-RIPENING.
RESULTS ARE EXPRESSED IN PERCENT OF THE TOTAL NITROGEN OF THE RESPECTIVE CHEESES OF THE SAME "MAKE" AT THE AGE HERE DEFINED.

Soluble protein nitrogen.

Subtraction of the *soluble nitrogen* (*W. and B.*), the non-protein nitrogen from the soluble nitrogen of the same cheese, gives what may be termed the *soluble protein nitrogen*. From the determinations on the cheese of the day of making the soluble protein nitrogen is seen to be 7·47 per cent. of the total nitrogen and 60·5 per cent. of the soluble nitrogen. This relationship to the total nitrogen prevails on the whole for the first 8 days of ripening. About

Table II. *Nitrogen distribution in Kingston cheese-ripening.*

Age of cheese in days	Soluble protein nitrogen		Protein coagulable by heat and acetic acid		Protein not coagulable by heat and acetic acid but precipitable by trichloracetic acid	
	% of total nitrogen	% of soluble nitrogen	% of total nitrogen	% of soluble protein nitrogen	% of total nitrogen	% of soluble protein nitrogen
Day of making	7·47	60·50	5·22	69·88	2·25	30·12
1	7·66	56·33	4·90	63·97	2·76	36·03
2	11·23	61·13	7·85	69·90	3·38	30·10
4	8·09	52·12	4·26	52·65	3·83	47·35
5	8·32	50·09	5·83	70·07	2·49	29·93
6	8·74	50·81	7·62	87·18	1·12	12·82
7	8·32	46·43	7·46	89·66	0·86	10·34
8	9·30	49·81	8·29	89·14	1·01	10·86
9	7·81	44·55	4·91	62·87	2·90	37·13
11	5·31	29·53	4·06	76·46	1·25	23·54
21	7·92	35·15	6·10	77·02	1·82	22·98
28	5·88	25·53	3·69	62·75	2·19	37·25
35	.	.	.	.	2·46	.
42	6·27	23·87	2·67	42·58	3·60	57·42
49	6·84	25·59	2·69	39·33	4·15	60·67
63	9·82	30·02	4·50	46·07	5·32	53·93
84	6·54	19·66	3·18	48·62	3·36	51·38
98	5·95	17·93	2·83	47·56	3·12	52·44

Soluble protein nitrogen = soluble nitrogen minus soluble nitrogen (W. and B.).
Protein coagulable by heat and acetic = soluble nitrogen minus soluble nitrogen (O.-J.).
Protein not coagulable by heat and acetic but precipitable by trichloracetic acid = soluble nitrogen (O.-J.) minus soluble nitrogen (W. and B.).

The results expressed in percentage of total nitrogen are given as percentage of the total nitrogen of the respective cheese of the same "make" at the age here defined. The results expressed in percentage of soluble nitrogen and of soluble protein nitrogen are calculated as percentage of the soluble nitrogen and of the soluble protein nitrogen respectively of the respective cheese (Table I).

the 11th day, however, a change in the relationship is to be observed; the soluble protein nitrogen is now but 5·31 per cent. of the total nitrogen. From the 1st day of ripening the soluble protein nitrogen in per cent. of the soluble nitrogen has been gradually declining until at the 11th day it is 29·53 per cent. of the soluble nitrogen. As the cheese ripens further the soluble protein nitrogen figure remains relatively constant although the soluble nitrogen content has risen from 17·98 per cent. (11th day) to 33·19 per cent. (98th day) of the total nitrogen—the soluble protein nitrogen being 5·95 per cent. of the

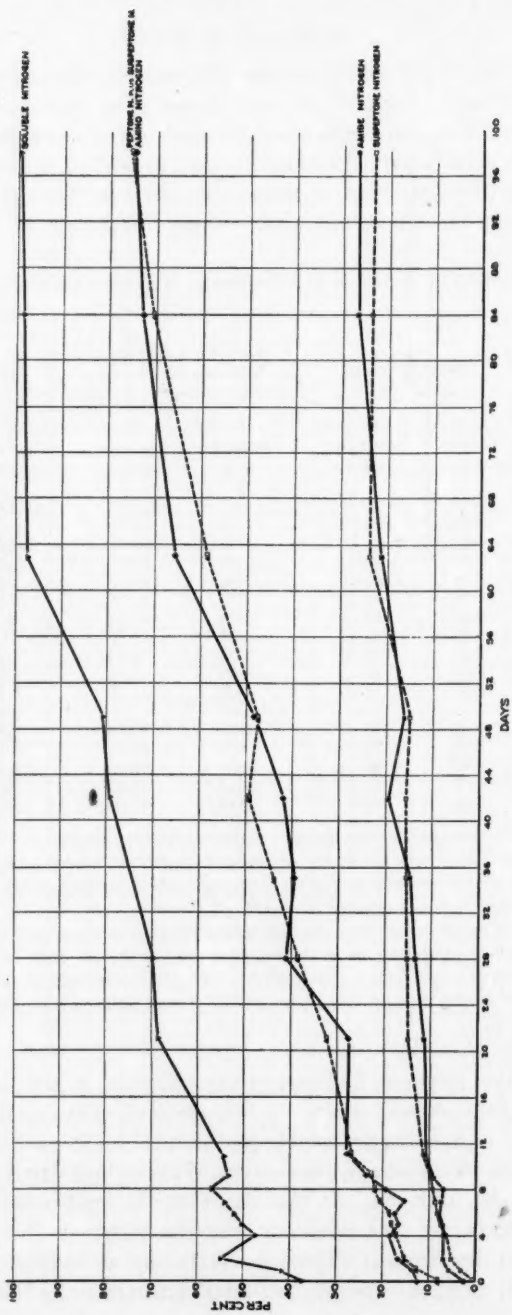

FIGURE 2.—NITROGEN DISTRIBUTION IN KINGSTON CHEESE-RIPENING.

THE RESULTS ARE CHARTED AS PER CENT OF THE MAXIMUM SOLUBLE NITROGEN FIGURE —— 33.19 (TABLE I.)

total nitrogen and but 17·93 per cent. of the soluble nitrogen at the 98th day. It is to be seen that an equilibrium between the amount of soluble protein undergoing proteolysis and the amount of protein becoming soluble has been established.

Although on the whole the difference between the results of the soluble nitrogen determinations by the method of Orla-Jensen and by the method of Wasteneys and Borsook on respective cheese, is, in general, of the same order of magnitude, it is to be seen in Tables I and II, that on the 6th, 7th and 8th days of ripening the results obtained by both methods approximate one another. On the other hand, when considering the results of the soluble nitrogen and soluble nitrogen (O.-J.) determinations the maximum differences throughout the 98 days are shown on the 6th, 7th and 8th days of ripening. Necessarily it follows that on those days of ripening there is present in the cheese the maximum percentage of heat and acetic acid coagulable soluble protein and the minimum percentage of soluble protein not coagulable by heat and acetic acid but precipitable by trichloracetic acid.

It appears that the soluble protein nitrogen is quite different in quality at various periods of the ripening of the cheese—the difference being particularly marked in the cheese when 6, 7 and 8 days old. It may be that these differences in the distribution of soluble protein nitrogen bear some relation to the characteristic quality of Kingston cheese when 10–12 days old.

Amino nitrogen and amide nitrogen.

The *amino* nitrogen is that nitrogen unprecipitated by phosphotungstic acid from the soluble nitrogen (O.-J.) solution. The *amide* nitrogen, as defined, is that nitrogen of the protein decomposition products of the cheese not precipitated by phosphotungstic acid from the soluble nitrogen solution, *i.e.* under different conditions of concentration of the reagents. Possibly, in view of the methods of procedure followed, the amino nitrogen and amide nitrogen results might be expected to approximate one another. They do not do so. It will be seen on reference to Table I that the *amide* nitrogen maintains a roughly constant ratio to the *amino* nitrogen, being about one-third of the latter throughout the course of ripening.

In drawing attention to this most interesting relationship, we find it difficult to discern a reason for the difference. A possible explanation is that in the case of the amide nitrogen determination, phosphotungstic acid precipitates, along with the soluble proteins of the soluble nitrogen solution, considerable non-protein-nitrogenous compounds which escape precipitation under the conditions of the phosphotungstic acid precipitation of the soluble nitrogen (O.-J.) solution.

The validity of this statement is established in a most striking manner when the data secured by the method of Wasteneys and Borsook are applied to the interpretation of the amino and amide nitrogen results.

Proteose nitrogen, peptone nitrogen and sub-peptone nitrogen.

The soluble nitrogen of Wasteneys and Borsook is made up entirely of non-protein-nitrogenous compounds, hence the *soluble nitrogen* (*W. and B.*) may be referred to as the *non-protein nitrogen* (*N.P.N.*). This consists of compounds of varying complexity, grouped under the terms proteoses, peptones and sub-peptones. These cleavage products depend for their classification upon whether or not they are precipitated by certain substances under defined conditions—such conditions as govern the Wasteneys and Borsook procedure for the analysis of an incomplete protein hydrolysate.

The results thus obtained show the distribution of the proteose, peptone and sub-peptone nitrogen fractions during the cheese-ripening process: not only do the results show the distribution of the fractions defined but also the relative extent to which these cleavage products are to be found as the hydrolysis of the cheese protein proceeds.

The analytical data are tabulated in Table I and, in Fig. 1, expressed graphically in percentage of the total nitrogen of the respective cheese. So that the nature of the proteolytic breakdown may be the more clearly seen, the variations in the soluble nitrogen (W. and B.)—(non-protein nitrogen)—obtained by calculations based on the data in Table I and expressed in percentage of the maximum soluble nitrogen (W. and B.) are plotted in Fig. 3. Similarly in Fig. 3 are shown the variations in the amount of proteose, peptone and sub-peptone nitrogen fractions respectively, in percentage of the maximum soluble nitrogen (W. and B.). It may be observed that the soluble nitrogen (W. and B.) curve of Fig. 3 is really a "percentage hydrolysis curve."

Before proceeding to a detailed study of the nitrogen distribution picture portrayed in Fig. 3, consideration must be given to the differences between the *amino* and *amide* nitrogen results. In the light of the results obtained by the Wasteneys and Borsook fractionation it is seen that the amide and amino nitrogen values may be correlated with the sub-peptone and sub-peptone plus peptone nitrogen respectively. In order that the results obtained by different methods of analysis may be compared, it is essential that they be expressed in terms of a common factor. In Fig. 2 the amide nitrogen, amino nitrogen, sub-peptone nitrogen, and sub-peptone nitrogen plus peptone nitrogen are graphed in percentage of the soluble nitrogen. The superimposed curves in Fig. 2 speak for themselves.

The curves for the proteose, peptone and sub-peptone nitrogen fractions should be considered, not only in terms of the maximum soluble nitrogen, but also in terms of the soluble nitrogen at whatever point along the curves it is desired to make comparison.

Following the curves, it is to be seen that in the 3-hour old cheese the soluble nitrogen is made up of proteose and peptone nitrogen, rather more of the latter, and no sub-peptone nitrogen. Within 24 hours the proportion changes, however, for the sub-peptone nitrogen fraction now appears. By the

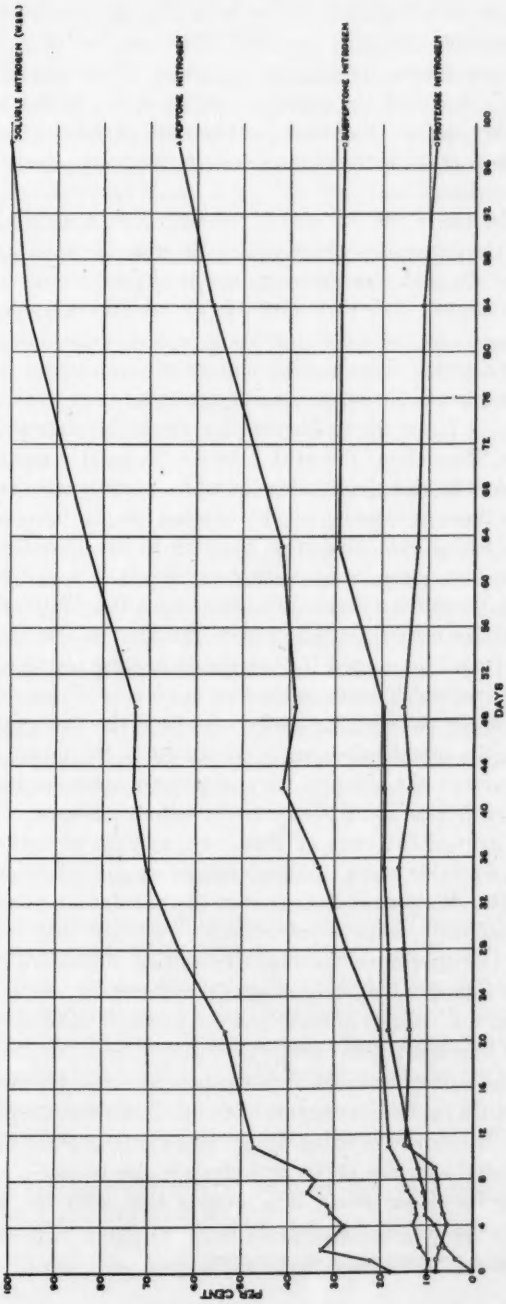

FIGURE 3—NITROGEN DISTRIBUTION IN KINGSTON CHEESE-RIPENING

THE RESULTS ARE CHARTED IN PERCENT OF THE MAXIMUM SOLUBLE NITROGEN (W & B) FIGURE—27.24 (TABLE 1)

4th day is shown a decline in the proteose nitrogen coincident with a definite rise in the soluble nitrogen; obviously, therefore, less of the soluble nitrogen now exists as proteose: the amount of peptone nitrogen has slightly increased, but again, in terms of the soluble nitrogen, it constitutes a less proportion than was the case on the initial day: the sub-peptone nitrogen fraction has increased to such an extent that it now forms a considerable portion of the soluble nitrogen.

From the 4th to the 9th day of ripening there is a definite though slight increase in the proteose, peptone and sub-peptone nitrogen fractions. Between the 9th and the 11th day the sharp rise in the soluble nitrogen (W. and B.) content is reflected more particularly in the amount of proteose nitrogen and, to some degree, in the peptone and sub-peptone nitrogens. During the next 10 days of ripening there is little change in the nitrogen distribution. But from the 21st to the 35th day of ripening a steady increase in soluble nitrogen (W. and B.) is observed, an increase for which the peptone nitrogen alone is responsible. Throughout the next 2 weeks the soluble nitrogen (W. and B.) remains constant, yet the distribution of its parts is measurably altered, the peptone nitrogen increasing at the expense of the proteose nitrogen. The period of ripening extending from the 49th to the 63rd day is characterised by a steady rise again in the soluble nitrogen (W. and B.), an increase for which the sub-peptone nitrogen is largely responsible. This forms an interesting contrast to the period extending from the 21st to the 35th day when the peptone nitrogen accounted for the rise in soluble nitrogen. The rise in the soluble nitrogen which began on the 49th day is steadily maintained throughout the remainder of the ripening period. As from the 63rd day this progressive increase in the soluble nitrogen is attributed to the increase in the peptone nitrogen fraction, the sub-peptone and proteose nitrogen fractions remaining constant yet decreasing relatively to the soluble nitrogen.

In the light of the work of Wasteneys and his co-workers (10, 11) on pure proteins and specific enzymes, the classification and definition of the proteases by Willstatter, Waldschmidt-Leitz *et al.* (12), the data appearing in a study of the casein-splitting abilities of certain lactic acid bacteria in chalk milk culture (13) and studies on the sugar-fermenting abilities of certain lactic acid bacteria as influenced by defined nitrogen sources (14), we suggest that at the beginning of the ripening of the cheese the protein hydrolysis is of a peptic-like nature and that this peptic-like proteolysis is to be attributed, not only to the action of the rennet, but also, as our papers about to appear will show, to the elaboration of a peptic-like enzyme by certain lactic acid streptococci. But within the first 24 hours of ripening the amount of sub-peptone appearing suggests that associated with the peptic hydrolysis is also tryptic-like action (14).

The results of our study thus suggest that after the first few hours of ripening the proteolytic breakdown in the ripening of Kingston cheese is of an associative peptic-tryptic-like nature.

SUMMARY.

A study has been made of the nitrogen distribution in Kingston cheese, over a ripening period of 98 days. For the determinations, methods evolved and employed by Orla-Jensen, Barthel and others have been drawn upon. In addition, a departure has been made in applying to this study the method developed by Wasteneys and Borsook for the fractional analysis of incomplete protein hydrolysates.

Determinations were carried out on cheese of the same "make" from the day of making to the 98th day of ripening at intervals defined and recorded in the tables and figures.

Results obtained when employing the methods of Orla-Jensen and of Barthel have been complemented by the application of the Wasteneys and Borsook method, which defines the nature and the amount of various protein decomposition fractions (proteose, peptone and sub-peptone) formed as the ripening of the cheese proceeds.

Particularly striking is the finding that the difference in the results of the amide nitrogen determinations and the amino nitrogen determinations can be explained when interpreted in the light of data secured by the application of the method of Wasteneys and Borsook. The amide nitrogen curve has been found to coincide with the sub-peptone nitrogen curve, and the amino nitrogen curve to superimpose itself upon the curve depicting the sum of the sub-peptone nitrogen and the peptone nitrogen.

The results of our study, subject to qualification as further data on the nature of specific enzymes may appear, suggest that after the first few hours of ripening the proteolytic breakdown in the ripening of Kingston cheese is of an associative peptic-tryptic-like nature.

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37. STUDIES IN CHEDDAR CHEESE

I. THE OXIDATION-REDUCTION POTENTIALS OF RIPENING CHEDDAR CHEESE

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(With 2 Figures.)

The work described in this paper is the first part of a scheme of investigation into the fundamental biochemistry and bacteriology of Cheddar cheese ripening, which has been planned at this Institute. It is hoped to co-ordinate the work with that already being carried out at other centres in the Empire.

Introduction.

One of the fundamental points of interest from the biochemical point of view is the oxygen tension in the interior of a cheese. It is known that a certain amount of carbon dioxide and hydrogen are formed as the result of bacterial metabolism, but the exact extent to which the oxygen disappears is not known. Any method of estimating the oxygen tension in the interior of a cheese would be attended with considerable experimental difficulty and probable error, but the trend of the work in the last decade has indicated that the oxidation-reduction potential of a medium may be more significant from a biological point of view than the molecular oxygen tension. Attention was therefore directed to this aspect of the problem.

Oxidation-reduction potentials may be considered either in terms of E_h obtained by direct electrode measurement, or in terms of rH, or hypothetical hydrogen pressures. Just as ($H^{\cdot}$) is for convenience stated as pH, so P or the hypothetical hydrogen pressure is stated in terms of rH, where $r\mathrm{H} = \log 1/P$. The relation between rH and E_h in ideal systems $\left(r\mathrm{H} = \frac{E_h}{0{\cdot}029} + 2p\mathrm{H}\right)$ does not hold for all systems or over all pH ranges in practice. Nevertheless, since the work described in this paper was carried out over a narrow pH range, it may be assumed that a constant relationship exists between the rH and E_h of the systems studied (1).

Typical pH values of the cheese studied are given in Table I. The values were obtained from a number of cheese from 5 to 5½ months old, using the quinhydrone electrode standardised in $M/20$ potassium hydrogen phthalate buffer at pH 3·97.

Table I. p*H values of cheese determined by the quinhydrone electrode.*

5·00	5·12	5·20	5·30	5·42
5·02	5·15	5·23	5·30	5·44
5·05	5·15	5·23	5·30	
5·05	5·19	5·24	5·30	
5·08	—	5·25	5·30	
		5·25	5·35	
		5·27	5·37	
		5·27		
		5·27		
		5·28		
		5·28		
		5·28		

Mean value 5·23.

Actual values obtained with the present experimental cheese in the earlier days of ripening are given in Table II.

Table II.

Cheese No.	Age in days	*p*H
7	1	4·90
5	8	5·07
6	14	5·18
4	16	5·15
1	17	5·10

The significance of oxidation-reduction potential in bacterial metabolism.

The oxidation-reduction potential or reducing intensity of a medium may be considered both from the point of view of the effect of the *r*H on the growth of bacteria and from the aspect of the *r*H induced by the growth of bacteria.

The effect of the potential of the medium on bacterial growth.

The phenomena of allelocatalysis(2, 3), the bacteriostatic action of media exposed to light(4) and the influence of cysteine (0·1–0·8 per cent.) in promoting anaerobic growth(5) may all be interpreted in terms of oxidation-reduction potential.

It has recently been shown that the spores of *Cl. tetani* will not germinate in a medium at an E_h greater than 0·11 v. at *p*H 7 (*r*H = 18)(6).

The *r*H ranges of growth for bacteria generally have been given as from 0 to greater than 20 for facultative anerobes, and from 0 to 12 for strict anaerobes(7).

The effect of bacterial growth on the potential of the medium.

It is known that all types of micro-organisms tend to adjust the oxidation-reduction potential of their medium to a definite zone which usually changes during the successive phases of growth, and that growth can only occur between certain limits. These facts are of fundamental importance not only to the bacteriologist but to the dairy industry. Thus the auto-sterilisation of some streptococci, pneumococci and other organisms has been shown to be due to the formation of peroxide. The addition of catalase destroys the peroxide and

permits fresh growth(8). The occurrence of oiliness in milk and other products due to copper acting both as a peroxide producer and as a peroxidase, or induced by light, is controlled by the oxidation potential of the milk as determined by the flora. A flora consisting of yeasts and cocci will in general not significantly reduce the E_h and so not influence the oxidising intensity produced by the catalytic action of copper or light. Coliform organisms, streptococci and other bacteria inducing a low E_h will prevent oiliness, however, by removing the milk from the zone of rH over which fat oxidation is possible(9).

Bacteria may control such oxidative reactions not only by removing molecular oxygen by their respiratory processes but by producing, usually by fermentative reactions, systems capable of inducing a high reducing intensity (or low E_h) in the medium. It is not necessary that such systems be reversible; non-reversible systems (*i.e.* those involving a change more complex than the transfer of free electrons) in which intermediate reactions may be reversible, may have a chemical and biological significance, and affect the oxidation-reduction potential of the medium.

The fault known as "rusty spot" in hard cheese has been shown to be controlled by the oxidation-reduction potential of the medium, pigment apparently only being produced by the causative organism at an rH less than 7. Vigorously growing associated bacteria have been shown either to stimulate or to inhibit the formation of pigment according to the rH induced by them in the medium(10). Thus rusty spot would presumably not appear in a cheese if the rH were maintained above 7.

The manufacture of Cheddar cheese is a process which results in the establishment of an almost pure culture of *S. lactis* (99 per cent.) followed later by lactobacilli. Hence the oxidation-reduction potential of a Cheddar cheese should be that of a culture of *S. lactis*, and only those bacteria able to grow at this rH will constitute the flora of Cheddar cheese, at any rate in the earlier stages of making and ripening.

DISCUSSION OF METHODS.

Three methods of following the values of the potential of ripening Cheddar cheese were available (1931).

(1) By the immersion of gold or platinum electrodes in the cheese itself, making contact by means of an agar bridge dipping into saturated KCl held in a cavity in the rind of the cheese, and measuring the potential against a standard half cell (Fig. 1). Attempts were made to measure the E_h values of three cheese by this method. Erratic values were obtained and it was discovered that "creep" had taken place in all cases. The electrodes used had been tested by the ordinary nitric acid method, which failed to show defects during the ordinary period of immersion. (Electrodes are now examined in this laboratory by immersion in 50 per cent. nitric acid for a fortnight, the mercury then poured off and the wire inside the glass tube examined with a lens. The

slightest trace of mercury salt is sufficient to condemn the electrode. Owing to the difficulty of making a perfect junction between Pyrex glass and platinum, soft glass is now used.)

(2) By the indicator micro-injection method(11).

(3) By incorporating an rH indicator in the milk at the time of making and boring the cheese at frequent intervals.

The uncertainty of the effect of fat on the electrode together with the difficulty of obtaining electrodes free from "creep" and the possible errors and complications of the micro-injection method warranted the investigation of the possibilities of the third method.

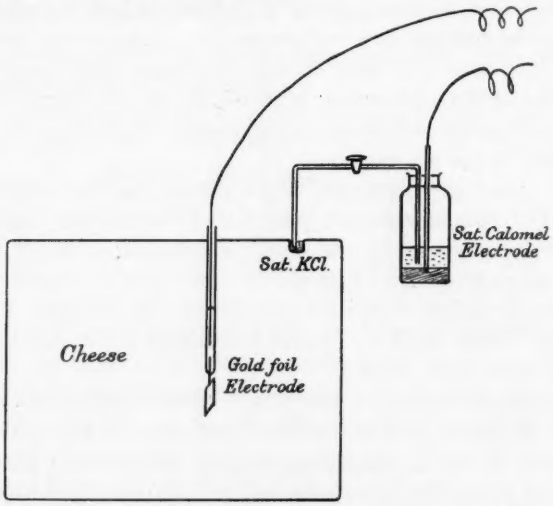

Fig. 1.

Choice of indicators.

Cultures of *S. lactis* in milk have been found to induce an E_h of about $-0{\cdot}2$ v.(1, 9). Since a cheese has a pH of about 5, this would be equivalent to an rH of about 3 (assuming the usual formula $r\text{H} = \frac{E_h}{0{\cdot}029} + 2p\text{H}$).

Indicators having the rH values shown in Table III were therefore chosen.

Table III.

	rH for		
	99 % reduction	50 % reduction	1 % reduction
Methylene blue	12·4	14·4	16·4
Janus green (1st stage)	11·0	13·0	15·0
,, (2nd ,,)	3·0	5·0	7·0
Neutral red	1·7	3·7	5·7
"Phenol safranine"	−5·5*	−3·5*	−1·5*

* Owing to the fact that the behaviour of this dye has not been thoroughly investigated, these values are merely tentative(11).

Manufacture of cheese.

The cheese were made by experienced makers of experimental cheese and with the object of making cheese as close to the true Cheddar type as possible. The milk used was from the Institute's herd (Grade A, T.T. standard), and its production and transport were controlled. The data for the experimental cheese are given in Table IV. Cheese of 20 lb. weight were made because small cheese (4–5 lb.) sometimes used for experimental work cannot be considered normal Cheddar, owing to the great influence of physical conditions which are different in small and in large cheese.

Table IV.

Cheese No.	Indicator used	Quantity of milk (gallons)	Age of milk on making (hours)	Bacterial count	Presence or absence of *B. coli* (1 c.c. to $\frac{1}{1000}$ c.c.)	Quantity of indicator (g.)	Stage at which the dye was first reduced
1	Janus green	20	3–16	< 5,000	—	1	Cheddaring (1st), interior of curd (2nd)
4	Neutral red	20	3–16	< 5,000	—	2	Interior of curd
6	Safranine	20	3–16	1,000	—	1	
2	Janus green	20	3–16	500	—	0·5	Pitching (1st)
5	Neutral red	20	3–16	< 5,000	—	1	Cheddaring
7	Safranine	20	3–16	< 5,000	—	0·5	
3	Janus green	20	3–16	10,000	—	0·25	Pitching
8	Methylene blue	20	3–16	< 5,000	—	0·25	Pitching

Method of incorporating the indicator.

The indicator was added in 1 per cent. aqueous solution to the milk and thoroughly stirred in just before rennetting.

GENERAL OBSERVATIONS.

It was observed that the higher the bacterial count of the milk and the lower the concentration of the indicator, the sooner did reduction take place. The reducing intensity was sufficient after pitching to reduce Janus green (rH 13) on the surface of the curd. After Cheddaring, neutral red (rH 4) was also reduced against the oxidising tendency of the air. Safranine was not reduced during the process of manufacture.

Incidentally it was observed quite independently by the cheese-makers that when sunlight fell on the curd the reduction was greatly accelerated. This is an exact analogy of the bleaching of natural pigments observed in the curd by New Zealand workers(12).

Criteria of the cheese as typical Cheddar.

It is possible to criticise the technique of these experiments on the grounds that (1) the cheese were affected, bacteriologically at least, by the dye, and (2) the indicators did not remain in the cheese as reversible systems.

Possible toxic effect of the dyes.

The first criticism that the cheese might be affected by the presence of the dye is answered by the fact that neither of the cheese-makers noticed anything abnormal in the process of manufacture apart from the colour imparted by the indicator. Acidities developed normally although the usual titrations could not be carried out because of the presence of the dyes. Further the ripening process was in every way normal, the textures and flavours appearing in the usual sequence as the cheese ripened. The cheese were examined by an expert grader, who was unaware of the nature and objects of the experiment, and who passed the cheese as normal in every way apart from the colour. It may be assumed therefore that in the concentrations used the dyes had no effect on the flora or ripening of the cheese.

The grader's scoring of the cheese is given in Table IX.

Table IX.

Cheese No.	Indicator	Grader's remarks
1	Janus green	Very nice cheese, flavour good
4	Neutral red	Good texture, fair flavour
6	Safranine	Open texture, slightly bitter
2	Janus green	Very nice cheese, mild clean flavour
5	Neutral red	Good cheese, crumbly and hard
7	Safranine	Rather dry, clean flavour
3	Janus green	Good flavour and texture, soft
8	Methylene blue	Good flavour, slightly open in texture, good quality

General remarks. All were pronounced to be typical Cheddar cheese of good quality.

As further evidence that the cheese were normal, the bacteriological examinations of the cheese at the termination of the experiment are given in Table X. One gram quantities taken from various parts of the cheese were emulsified in 99 c.c. saline at 45° C. and the dilutions plated in the usual way.

Table X.

Cheese No.	Indicator used	Total count (Yeast extract dextrose agar)	Coliforms (Bile salt lactose)	Liquefiers (Gelatine)	Miscellaneous (Nutrient agar)
1	Janus green	8,000,000	<100	<100	12,000
1	Neutral red	9,600,000	<100	<100	Pin points only
1	Safranine	5,000,000	<100	<100	,,
2	Janus green	120,000	<100	<100	20,000
2	Neutral red	910,000	<100 (A.R. in 1/100 c.c.)	<100	Pin points only
2	Safranine	7,300,000	<100 (A. in 1/100 c.c.)	<100	,,
3	Janus green	4,800,000	<100 (A.R. in 1/100 c.c.)	<100	,,
1	Methylene blue	1,500,000	<100	300	90,000

A. = acid, R. = reduction.

The figures call for but little comment, conforming to those obtained by other workers. The low counts obtained for coli, liquefiers and miscellaneous organisms (*i.e.* non-lactic acid bacteria) are typical of those for good quality Cheddar cheese.

Chemical action on indicators.

The criticism that the indicators might have undergone chemical change is answered by the fact that on boring or cutting cheese containing a reduced dye, the colour rapidly reappeared. Safranine reoxidised in a few seconds, Janus green and methylene blue in a few minutes and neutral red in a somewhat longer time. It is safe therefore to assume that a significant amount of the dyes remained chemically unaltered. The behaviour of the indicators in the cheese is shown in Table V.

Table V.

Indicator used	Colour when reduced	Colour changes	Colour when in equilibrium with the air
Janus green	Colourless	Pink-mauve to deep lilac	Bluish purple
Neutral red	Mustard yellow	Pink to deep orange	Deep reddish orange
Safranine	Colourless	Faint pink to bright pink	Bright red or deep pink
Methylene blue	Colourless	Yellow to green	Bright blue-green

Two points call for comment. Neutral red possessed a considerable lag period in showing reoxidation compared with Janus green. Janus green, when reduced to the pink form, is not reoxidised by molecular oxygen, but partial reoxidation to the blue form took place under the influence of cheese. (As a matter of interest, it may be mentioned that sterilised milk is unable to bring about this reoxidation.)

The observations made upon boring the cheese are recorded in Tables VI–VIII. The colours were observed immediately the borings were withdrawn.

The two cheese made with methylene blue remained quite colourless throughout the period of ripening (208 days). It may therefore be assumed that the rH remained below 12·4. The depth of the oxidised zone varied from 1/16 in. to 1/8 in.

Discussion of results of clean milk cheese.

The indicators giving most information about the reducing intensities in ripening Cheddar cheese made from clean milk are Janus green, neutral red and safranine.

It is evident that good agreement is obtained between Janus green and neutral red which both indicate an rH of about 2 in ripening cheese. Safranine indicates a somewhat lower value, but in view of the lack of accurate data for this dye the discrepancy is not serious. Of the three, safranine is the most toxic and has been the least studied, so that more weight may be attached to the results obtained with the other indicators. It is not intended to attach any precise meaning to the rH values given; they are only regarded as of comparative value. The results obtained may be summarised as follows:

(1) Ripening Cheddar cheese maintain internally reduction potentials approximately corresponding to almost complete reduction of Janus green and

Table VI.

Cheese 1.
Dye added, Janus green (1 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	*r*H
1	Cream	$\frac{1}{8}$	<3
3	,,	$\frac{1}{8}$	<3
4	,,	$\frac{1}{8}$	<3
12	Faint pink	$\frac{1}{8}$	3
15	,,	$\frac{1}{8}$	3
17	Faint lilac	$\frac{1}{8}$	3
21	Faint pink	$\frac{1}{8}$	3
24	,,	$\frac{1}{8}$	3
29	Faint lilac	$\frac{1}{8}$	3
33	,,	$\frac{1}{8}$	3
36	,,	$\frac{1}{8}$	3
44	Dirty cream	$\frac{1}{8}$	<3
46	,,	$\frac{1}{8}$	<3
49	,,	$\frac{1}{8}$	<3
51	,,	$\frac{1}{8}$	<3
54	Faint pink	$\frac{1}{8}$	3
59	,,	$\frac{1}{8}$	3
71	Creamy	$\frac{1}{8}$	<3
74	,,	$\frac{1}{8}$	<3
81	,,	$\frac{1}{8}$	<3
88	Faint pink	$\frac{1}{8}$	3
95	,,	$\frac{1}{8}$	3
112	Creamy	$\frac{1}{8}$	<3
142	,,	$\frac{1}{8}$	<3
172	Faint pink	$\frac{1}{8}$	3
203	Creamy	$\frac{1}{8}$	<3
231	Faint lilac	$\frac{1}{4}$–$\frac{1}{2}$	3

Cheese 2.
Dye added, Janus green (0·5 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	*r*H
1	Colourless	$\frac{1}{16}$	<3
5	,,	$\frac{1}{8}$	<3
7	Faint pink	$\frac{1}{8}$	3
8	,,	$\frac{1}{8}$	3
14	Colourless	$\frac{1}{8}$	<3
16	Creamy	$\frac{1}{8}$	<3
17	Faint lilac	$\frac{1}{8}$	3
19	Creamy	$\frac{1}{8}$	<3
22	,,	$\frac{1}{8}$	<3
26	,,	$\frac{1}{8}$	<3
28	Colourless	$\frac{1}{8}$	<3
31	Creamy	$\frac{1}{8}$	<3
34	Colourless	$\frac{1}{8}$	<3
38	,,	$\frac{1}{8}$	<3
43	,,	$\frac{1}{8}$	<3
51	,,	$\frac{1}{8}$	<3
63	,,	$\frac{1}{8}$	<3
66	,,	$\frac{1}{8}$	<3
73	,,	$\frac{1}{8}$	<3
80	,,	$\frac{1}{8}$	<3
87	,,	$\frac{1}{8}$	<3
104	,,	$\frac{1}{8}$	<3
134	,,	$\frac{1}{16}$	<3
164	,,	$\frac{1}{8}$	<3
195	,,	$\frac{1}{8}$	<3
223	Faint lilac	$\frac{1}{8}$	3

Cheese 3.
Dye added, Janus green (0·25 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	*r*H
1	Colourless	$\frac{1}{16}$	<3
2	,,	$\frac{1}{16}$	<3
3	,,	$\frac{1}{16}$	<3
4	,,	$\frac{1}{16}$	<3
8	Faint lilac	$\frac{1}{8}$	3
12	Creamy	$\frac{1}{8}$	<3
15	,,	$\frac{1}{8}$	<3
18	,,	$\frac{1}{8}$	<3
21	Colourless	$\frac{1}{8}$	<3
23	Creamy	$\frac{1}{8}$	<3
25	,,	$\frac{1}{8}$	<3
30	,,	$\frac{1}{8}$	<3
38	,,	$\frac{1}{8}$	<3
50	Colourless	$\frac{1}{8}$	<3
53	,,	$\frac{1}{8}$	<3
60	,,	$\frac{1}{8}$	<3
67	,,	$\frac{1}{8}$	<3
74	,,	$\frac{1}{8}$	<3
91	,,	$\frac{1}{8}$	<3
121	,,	$\frac{1}{8}$	<3
151	,,	$\frac{1}{8}$	<3
182	,,	$\frac{1}{8}$	<3
210	Creamy	$\frac{1}{4}$–$\frac{1}{2}$	<3

Table VII.

Cheese 4
Dye added, neutral red (2 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	rH
1	Pale orange	¼	3
2	Pale yellow	¼	2
3	"	¼	2
10	Pale orange	¼	3
13	Faint pink orange	¼	3
16	"	¼	3
19	"	¼	3
22	Deep orange	¼	3
27	"	¼	3
31	"	¼	3
34	"	¼	3
42	"	¼	3
44	Bright orange	¼	3
49	"	¼	3
57	Yellow orange	¼	2
69	Mustard yellow	¼	<2
72	"	¼	<2
79	"	¼	<2
86	"	¼	<2
93	"	¼	<2
110	"	¼	<2
140	"	¼	<2
170	"	¼	<2
201	"	¼	<2
229	"	¼	<2

Cheese 5.
Dye added, neutral red (1 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	rH
1	Buff	1/16	3
3	Bright yellow	¼	2
5	"	¼	2
6	"	¼	2
8	Orange	¼	3
12	"	¼	3
15	"	¼	3
17	"	¼	3
20	"	¼	3
24	"	¼	3
27	Deep orange	¼	3
30	"	¼	3
33	"	¼	3
37	Bright orange	¼	3
42	"	¼	3
50	Yellow orange	¼	2
62	Bright yellow	¼	<2
65	Mustard yellow	¼	<2
72	"	¼	<2
79	"	¼	<2
86	"	¼	<2
103	"	¼	<2
138	"	¼	<2
163	"	¼	<2
194	"	¼	<2
222	"	¼–½	<2

Table VIII.

Cheese 6.
Dye added, safranine (1 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	rH
1	Pale pink	1/16	−3
3	"	1/16	−3
8	Bright pink	¼	−2
14	"	¼	−2
17	Deep pink	¼	−2
20	"	¼	−1
22	"	¼	−1
25	"	¼	−1
29	"	¼	−1
32	"	¼	−1
35	"	¼	−1
40	"	¼	−1
42	"	¼	−1
47	"	¼	−1
55	"	¼	−1
67	"	¼	−1
70	"	¼	−1
77	"	¼	−1
84	"	¼	−2
91	"	¼	−2
108	"	¼	−2
138	"	¼	−2
168	Fainter pink	¼	−2
199	"	¼	−2
227	Bright pink	¼	−2

Cheese 7.
Dye added, safranine (0·5 g.)

Age in days	Colour of indicator	Depth of zone of oxidised form (in.)	rH
1	Faint pink	1/16	−3
5	"	¼	−3
6	"	¼	−3
8	Bright pink	¼	−2
9	"	¼	−2
10	"	¼	−2
13	Deep pink	¼	−2
17	"	¼	−2
20	"	¼	−2
23	"	¼	−2
26	"	¼	−1
30	"	¼	−1
35	"	¼	−1
43	"	¼	−1
55	Fainter pink	¼	−2
62	"	¼	−2
69	"	¼	−2
76	"	¼	−2
93	Deep pink	¼	−1
123	"	¼	−1
153	"	¼	−1
184	Fainter pink	¼	−2
212	"	¼	−2

neutral red and to the normal potential (50 per cent. reduction) of the phenol safranine.

(2) At the surface of the cheese and along borings and cracks in the cheese, zones of higher oxidation potential exist. It was found that the cheese to a depth of about $\frac{1}{8}$ in. was maintained at an *r*H above 14, and that a zone from $\frac{1}{4}$ in. to $\frac{1}{2}$ in. remained at an *r*H above 5.

(3) All three indicators in whose range the potentials of the cheese fell suggested a definite sequence in the potential of the cheese, irrespective of any absolute *r*H values. This is illustrated in Fig. 2, which gives the mean *r*H value for the seven cheese during the course of ripening. The following phases may be traced:

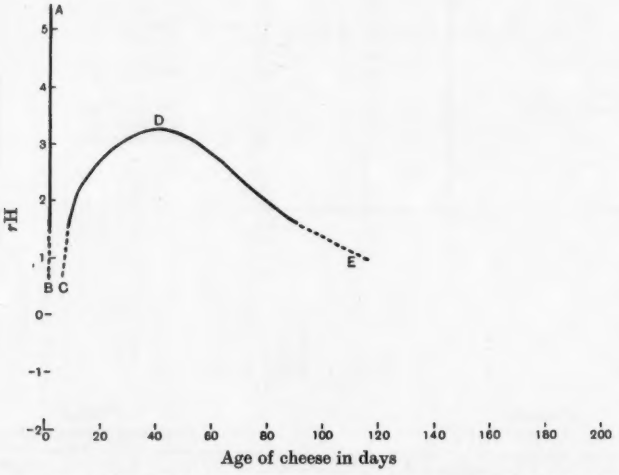

Fig. 2. Graph showing the approximate path of *r*H in ripening Cheddar cheese.

A–B (0 to 1 day) = period of initial reduction (growth of streptococci).
B–C (1 to 9 days) = period of maintenance of reducing conditions.
C–D (9 to 40 days) = period of oxidation (dying out of streptococci?).
D–E (40 to 200 days) = period of gradual reduction (growth of lactobacilli?).

(*a*) *From the time of manufacture until the end of the first day* (*A–B*). During this time the potential is steadily falling until it arrives at a value below that at which Janus green and neutral red are reduced and at which safranine is partly reduced. At this period the streptococci are increasing logarithmically and it may be assumed that the reducing intensity is caused by the products of growth of these bacteria, such as the trioses (*e.g.* methyl glyoxal) and acetaldehyde, which has been shown to possess an apparent potential(13).

(*b*) *From the first to about the ninth day* (*B–C*). The low potential is maintained, and after about the ninth day slowly rises. This period is characterised by the streptococcal count reaching a maximum and then falling steadily(15, 16).

Thus the maintenance of the strongly reducing conditions is apparently dependent on the number of viable bacteria.

(*c*) *From about the ninth day to about the fortieth day* (*C–D*). This is a period of slow reoxidation and is caused possibly by the gradual action of air diffusing into the cheese and increasing the concentration of oxidants in the cheese, the reducing action of the cells being gradually weakened.

(*d*) *From about the fortieth day to the end of the observed period* (200 *days*) (*D–E*). During this time there is a slight but definite reduction of the indicators, which is apparently due to the increase in the number of viable lactobacilli which are known gradually to replace the starter streptococci(14).

It was found that those lactobacilli preponderant in riper Cheddar cheese are able to reduce the litmus in litmus milk cultures, whereas those constituting the flora from the first to about the third month do not. The bacteriological aspects of cheese ripening will be discussed in a later communication.

Applications of the method to cheese research.

(1) The principle of the method used is, of course, that of the reductase test, which measures not only the number of viable organisms present, but their reducing intensities and capacities. Since the measurements described were taken over a long period of time, it may be assumed that the capacity factor (or that corresponding to the number of organisms present) does not appreciably affect the results. Differences in the rate of reduction were observed, however, in the cheese containing different amounts of the dye. The indicator colours give an approximate value of the *r*H or oxidation-reduction potential at different depths in the cheese and at different periods of ripening. Thus only those organisms capable of growing and multiplying at *r*H 3 will be able to flourish in the interior of the cheese. Strict aerobes and those requiring a medium at a higher potential will be unable to thrive. Different conditions hold however in a narrow zone (about $\frac{1}{4}$ in. wide) round the edge of the cheese and round cracks; this throughout the ripening is at a higher potential than the interior. It is known that many fault-producing organisms (*Proteus*, chromogenic cocci and various moulds) grow in this zone and on the outside of the cheese. The pale brown or pink zone seen in some over-ripe Cheddar cheese corresponds exactly with the oxidised zone of Janus green in cheese.

It may be observed, however, that the *r*H of these zones is still considerably below that at which oxidative taints, such as oiliness or cardboard flavour in milk, are known to occur. Thus these taints do not occur appreciably in milk maintained at an E_h of less than + 0·3 v. (*r*H 23 at *p*H 6·5)(9). It may therefore be predicted that such oxidative taints cannot occur in properly made Cheddar cheese and that the faults most serious and most difficult to eradicate will always be those of the "anaerobic" type, such as rusty spot and "stinkers."

It is obvious that conditions in this outer zone are different from those in the interior of the cheese, and therefore in the bacteriological examination of

cheese this zone should be considered separately, since counts obtained from samples containing portions of this zone will ascribe wrong values to the general cheese mass. The mass of cheese inside this zone, however, is apparently uniform in its oxidation-reduction potential.

(2) It may be possible to apply the principle as a reductase test on the cheese at different stages of ripening and thus to form the basis of estimation of the number of viable bacteria in the cheese. Since organisms differ in their ability to reduce various rH indicators, it may be possible to estimate the relative proportions of strong reducers such as *S. lactis* and weak reducers such as some *micrococci* by the use of different dyes.

(3) The growth of some extraneous organisms could be controlled by any mechanism capable of adjusting the potential of the cheese. Thus, for example, if the rH were maintained above 7 by means of oxidants, such as potassium nitrate, the rusty spot organism would be unable to form pigment and "rusty spot" would not appear in the cheese.

(4) It has been shown that the rH of Cheddar cheese follows a well-defined path within a certain range. Since chemical reactions are largely controlled by oxidation-reduction potentials, it follows that variations from the normal rH may lead to the formation of undesired substances or to the non-formation of desired substances.

The effect of oxidation-reduction potential on protein breakdown, undoubtedly the most important type of chemical reaction occurring in cheese-ripening, has not hitherto received much attention. The claim of Waldschmidt-Leitz(17) that the activator of certain proteolytic enzymes (kinase) is identical with the reduced form of glutathione, and that the oxidised form is inactive, may be of fundamental importance in the problem of cheese-ripening generally.

Summary.

1. A method is described for following the intensity of reduction (or oxidation-reduction potential) at different levels in the interior of ripening Cheddar cheese.

2. Variations in this potential throughout the course of ripening are described and mean values assessed.

3. Cheese are shown to be not homogeneous throughout their mass; outer zones of more highly oxidised condition exist near the surface of the cheese and round cracks and borings. The depth of these zones increases with the age of the cheese.

4. The values obtained can be correlated with the known bacteriological data for Cheddar cheese.

5. The significance of the data obtained in relation to cheese faults is discussed.

ACKNOWLEDGMENTS.

The author wishes to express his great indebtedness to Miss D. V. Dearden and Miss F. Chapman for making the cheese under such unusual conditions, to Mr A. Todd for inspecting and scoring the cheese, and to Dr A. T. R. Mattick for suggestions and encouragement throughout the work.

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38. THE INACTIVATION OF LIPASE IN DAIRY PRODUCTS BY TRACES OF HEAVY METAL SALTS

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(With 4 Figures.)

Lipases from various sources are inactivated by traces of heavy metals (Kastle(1), Haldane(2)). Thus pancreatic, liver and *Ricinus* lipases are poisoned by traces of silver, mercury and copper salts, the relative effects of the various metals differing greatly. Silver nitrate, for instance, is barely poisonous to liver lipase which is inactivated by traces of copper and mercuric salts.

Cow's milk, as drawn from the udder, contains a specific lipase capable of splitting milk fat under certain conditions (Rice(3)); thus the production of free fatty acids in some dairy products immediately after manufacture has been attributed to lipase action. The enzymatic liberation of fatty acids occurs to varying extents in butter made from unpasteurised cream and during the evaporation *in vacuo* (40° C.) of unpasteurised milk. The action of lipase is suppressed by low temperature storage and destroyed by pasteurisation at 62·5° C. and higher temperatures.

No work has been published on the effect of traces of heavy metal salts on the lipase associated with butterfat, though the processing of dairy products on a commercial scale causes some contamination of the product with the heavy metals used in the construction of dairy machinery and utensils (copper, iron, nickel, chromium, tin, aluminium). Some of these metals, even when found in traces in the processed product, have a profound effect on its keeping quality and cause various taints which are due to catalysed oxidation of butterfat.

During laboratory studies of the effects of traces of various metallic salts on the oxidation of the fat of butter care was taken to prevent as much micro-organic activity as possible, so butter made from sweet unpasteurised cream from milk of low bacterial count was used. It was found when working with samples of such butter in which traces of metallic salts with the normal amount of common salt had been incorporated that varying degrees of acidity as estimated by titration developed within a few days of manufacture, depending on the amount and character of the metal added. Since free acidity also had an effect in promoting fat oxidation, the effects of the various metals studied were therefore not strictly comparable in butter samples made from sweet cream. The development of acidity was due to lipase action, since no such

changes were evident in butter samples made from similar cream which had been pasteurised or in those made from unpasteurised cream and kept at 0–3° C. An opportunity was thus afforded of studying the effects of the various metals on the lipase in unpasteurised sweet cream butter.

The progress of peroxide formation in the various butter samples was also investigated by periodical determinations by the iodine liberation method of Lea(4) as modified by Taffel and Revis(5). It was found that, for the first few days, peroxide formation varied directly with the liberation of free fatty acid. That oleic acid oxidation (forming oleic peroxide) was the source of the peroxide has been established by the workers cited and by Greenbank and Holm(6). The ease with which oleic acid was liberated by lipase from fats pointed also to the oxidation of free oleic acid occurring first and not that of the combined acid radicle in the olein, since in samples from pasteurised cream there was no significant peroxide formation. In the circumstances, therefore, the rate of peroxide formation during the first few days after the manufacture of the butter, afforded another method of comparing the degrees of lipase activity in the differently treated butter samples.

DETERMINATION OF THE DEGREE OF LIPASE ACTION.

The determination of the liberated fatty acids is difficult when the lipase concentration or activity is low. The *detection* of free butyric acid, nevertheless, can be made with minute amounts. Thus Grossfeld and Battay(7) state that 1 part of butyric acid in 12,500 of material can be detected by smell, but this amount is too small to be determined by titration in small samples (5 g.) of butter.

Willstätter and Memmen(8) found that lipase had a relatively slight affinity for the lower esters and that pancreatic lipase, for instance, was 26,000 times more active towards olive oil than towards methyl butyrate. It was to be expected therefore that although the lower fatty acids of butterfat would be liberated only in sufficient amount to be detectable by smell, the higher fatty acids would be liberated in amounts determinable by titration. Unpublished work by the author has proved that oleic acid accounts for 60–70 per cent. of the total titratable value of the fatty acids liberated from butterfat. The volatile acids account for less than 5 per cent., so that oleic acid is liberated in 12–14 times their equivalent.

The titration method used throughout was that in which the fatty acids were titrated in an ethereal solution of the fat, using an underlying layer of 5 per cent. barium or calcium chloride solution to observe the end-point with phenolphthalein (Davies(9)). This method prevented products of proteolysis interfering with the titration value and minimised hydrolysis of soaps, since errors due to both causes enter when titrating in alcoholic solution.

Peroxide determination.

The peroxide of oleic acid, which is rapidly formed by the free acid in contact with atmospheric oxygen, was determined by the iodine-liberation method (Lea(4)). The daily increase in peroxide content of the butters was thus a relative measure of the free acidity during the first 4–5 days after manufacture. No such peroxide formation occurred in butter made from pasteurised cream during this period. After the first 4–5 days the catalytic action of the various metals included in the samples caused peroxide formation to occur generally.

Experimental procedure.

The butter was churned from unpasteurised sweet cream separated from clean milk, worked to the salting stage, and divided into ½ lb. portions. Salting with heavy metal-free A.R. sodium chloride at the rate of ¼ oz. per lb. was carried out, and traces of the heavy metal salts given below were incorporated in the salt in a known number of parts per million. (These are indicated by the figures following the metallic symbol):

copper lactate (Cu 2 and Cu 10);	iron lactate (Fe 5 and Fe 25);
cobalt acetate (Co 5 and Co 25);	nickel oleate (Ni 5 and Ni 25);
chromium acetate (Cr 5 and Cr 25);	tin oleate (Sn 10 and Sn 50);
aluminium butyrate (Al 10 and Al 50);	silver acetate (Ag 5 and Ag 50).

The salts were well incorporated into the butter.

Control samples of salted and unsalted butter from unpasteurised cream, salted butter from pasteurised sweet cream, and metalled samples from pasteurised cream containing Cu 2, Cu 10, Fe 5 and Fe 25, were also made. All samples were stored under the same conditions at room temperature (15–18° C.) in the dark. In all experiments, to facilitate accurate sampling, the samples were kept in wide-mouthed bottles so that softening of the butter to a semi-solid mass and thorough shaking until set, could be done. This treatment caused saturation with air at each treatment, a necessary procedure in securing uniform conditions for the subsequent study of the catalysis of butterfat oxidation in the same butter samples.

Acidity determinations were carried out daily at approximately the same hour on the control samples and on some of the other samples, most of which, however, were examined on alternate days (Table I). This was done over a period of 7 days, after which weekly determinations only were carried out. The latter results are not included here.

Discussion of results from the titration method.

On plotting the results given in Table I, it was found that the butter samples could be roughly divided into six groups, according to the degree of acidity developed. The "unpasteurised control" samples showed the maximum

development of acidity. The "pasteurised control," the "metalled pasteurised," and the Cu 10 samples showed the least development of acidity. No significant increases were observed in any of the samplings. The other samples developed acidity in varying degrees, and could be roughly classified into four groups. Representative curves of the acidity development with time for the different groups are given in Fig. 1. In all samples, almost constant acidity

Table I. *Titration values of acidities in various samples of butter (equivalent to c.c. N alkali per 100 g. butter) with age.*

No.	Sample	Age in days 0	1	2	3	4	5	6	7	Remarks
1	Control salted	0·80	1·45	1·50	1·72	1·80	2·00	2·06	2·10	—
2	Control unsalted	0·76	1·35	1·40	1·85	1·80	1·92	2·10	2·08	—
3	Cu 2	—	0·88	0·85	—	0·94	—	1·10	1·14	Fishy 4th day
4	Cu 10	0·81	—	0·78	—	0·95	—	0·93	0·93	Fishy 3rd day, tallowy 4th day
5	Fe 5	0·80	—	1·28	—	1·70	—	1·87	1·91	—
6	Fe 25	0·81	1·02	—	1·40	—	1·50	—	1·56	Fishy 5th day
7	Co 5	—	1·10	1·32	—	1·60	—	1·88	1·90	—
8	Co 25	0·83	0·96	—	1·20	—	1·32	—	1·35	—
9	Ni 5	—	0·99	1·26	—	1·59	—	1·90	1·86	—
10	Ni 25	0·84	0·92	—	1·10	—	1·18	1·30	1·30	Fishy 5th day
11	Mn 5	—	1·06	1·08	—	1·60	—	1·84	1·79	—
12	Mn 25	0·79	1·12	—	1·36	—	1·50	1·54	1·56	—
13	Cr 5	—	1·01	1·13	—	1·76	—	1·90	1·89	—
14	Cr 25	0·80	1·16	—	1·40	—	1·40	—	1·50	—
15	Ag 5	—	1·00	1·20	—	1·52	—	1·60	1·62	—
16	Ag 50	0·81	0·96	1·01	1·20	1·20	1·20	1·29	1·24	Tallowy 7th day
17	Sn 10	—	1·36	1·52	—	1·80	—	1·98	1·90	—
18	Sn 50	0·82	1·30	—	1·75	—	1·90	—	1·96	—
19	Al 10	—	1·40	1·40	—	1·90	—	2·04	2·00	—
20	Al 50	0·83	1·21	—	1·60	—	1·98	—	1·95	—
21	Control past.	0·76	0·80	0·80	0·86	0·90	0·86	—	0·89	—
22	*P* Cu 2	—	0·79	—	0·82	—	0·82	—	0·80	Fishy 4th day
23	*P* Cu 10	0·79	—	0·81	—	0·82	—	0·81	0·80	Fishy 3rd day, tallowy 4th day
24	*P* Fe 5	—	0·77	—	0·83	—	0·83	—	0·80	—
25	*P* Fe 25	0·81	—	0·83	—	0·84	—	0·80	0·82	Fishy 5th day

P=samples from pasteurised cream.

Table II. *The grouping of butter samples according to the lipase activity.*

Group	Grouped samples	Loss in lipase activity (%)
1	Control salted, control unsalted	0
2	Sn 10, Sn 50, Al 10, Al 50	5
3	Fe 5, Co 5, Ni 5, Mn 5, Cr 5	15
4	Mn 25, Fe 25, Cr 25, Ag 5	40
5	Co 25, Ni 25, Cu 2, Ag 50	70
6	Cu 10, past. control, *P* Cu 2, *P* Cu 10, *P* Fe 5, *P* Fe 25	100

development had occurred on the fifth day. If the maximum acidity development was that shown by the unpasteurised controls and the development of no acidity by the pasteurised samples was taken as complete absence of lipase action, the degree of inactivation of lipase by the metals of each group could be calculated. Table II gives the composition of each group in this respect with the percentage loss in lipase activity shown by each group.

Of the metals tested, traces of tin and aluminium caused the least amount of depression of lipase activity, and increasing the amounts of these metals 5 times did not increase the depression. Therefore it can be said that these metals had no significant effect on the lipase. But with the other metals tested, variation in the quantity of metal added caused a variation in the depression of lipase activity. Therefore the influence of these metals (groups 3 to 6) may be considered positive. Copper was the most potent of the tested

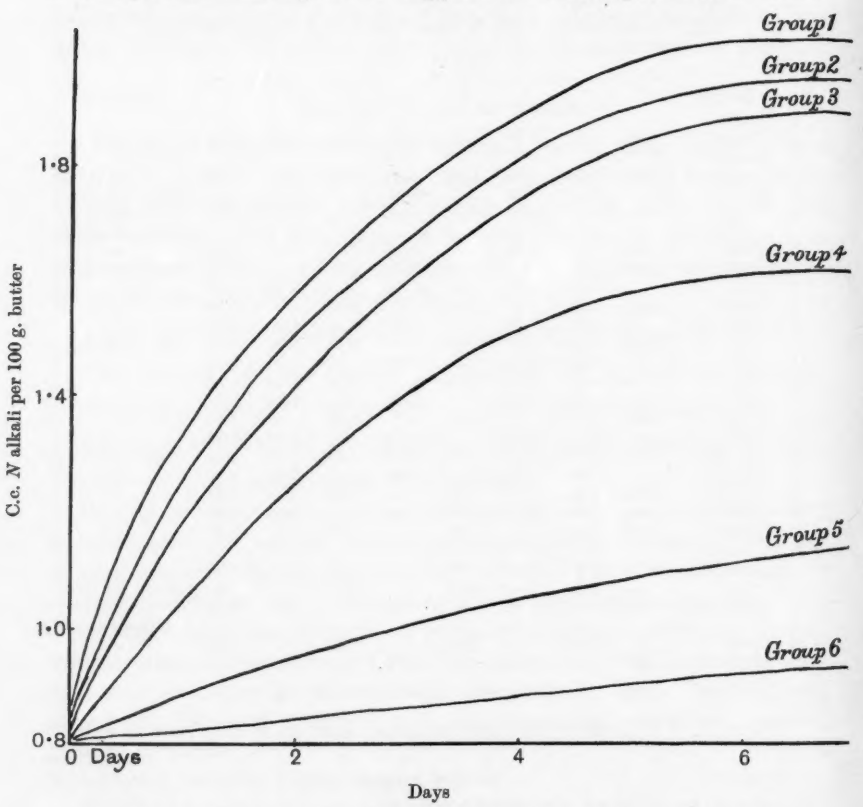

Fig. 1. Unpasteurised sweet cream butter. Development of acidity in samples containing traces of heavy metal salts. Temp. 15–18° C. For composition of groups see Table II.

metals, 10 parts per million completely inhibiting the action of lipase, while 2 parts per million caused a depression of 70 per cent. Cobalt and nickel were more powerful than iron, chromium or manganese. Although silver would be completely precipitated as silver chloride in the butter, it had a marked depressing effect, 50 parts per million showing a depression of 70 per cent. of the lipase activity.

The "metalled" samples of butter from pasteurised cream did not increase

in acidity during the observation period. Similar samples from unpasteurised cream and stored at 0–4° C. also did not develop acidity (Table III).

The amount of peroxide formation with increase in acidity.

Samples of butter from unpasteurised sweet cream were made which contained respectively 5 and 25 parts per million of iron (lactate) and 2 and 10 parts per million of copper (lactate). With the exception of group 2 (Table II) each group in that table was represented by one sample. One such batch of samples was kept in wide-mouthed bottles at room temperatures (15–18° C.)

Table III. *Acidities of butter samples* (*as c.c.* N *alkali per* 100 *g. butter*).

R.T. = room temperature storage (15–18° C.). I.C. = ice-chest storage.

		Day							
Sample		0	1	2	3	4	5	6	7
Control	R.T.	1·30	1·60	—	1·80	—	1·95	—	2·00
	I.C.	1·28	—	1·30	—	1·27	—	1·26	1·30
Fe 5	R.T.	1·27	1·51	—	1·70	—	1·80	—	1·88
	I.C.	1·32	—	1·30	—	1·29	—	1·32	1·33
Fe 25	R.T.	1·28	1·41	—	1·62	—	1·72	—	1·75
	I.C.	1·30	—	1·28	—	1·27	—	1·28	1·32
Cu 2	R.T.	1·30	1·30	—	1·35	—	1·40	—	1·45
	I.C.	1·29	—	1·30	1·35	1·28	—	1·32	1·28
Cu 10	R.T.	1·32	1·33	—	1·30	—	1·32	—	1·34
	I.C.	1·30	—	1·28	—	1·34	—	1·32	1·30

Table IV. *Peroxides in the butter samples* (*as c.c.* 0·002 N *thiosulphate per g. of butter*).

R.T. and I.C. as for Table III.

		Day							
Sample		0	1	2	3	4	5	6	7
Control	R.T.	1·0	2·0	3·0	3·8	5·5	5·8	6·0	6·0
	I.C.	0·9	1·0	—	0·9	—	0·8	—	1·0
Fe 5	R.T.	1·1	1·5	2·0	2·3	3·2	3·5	4·3	5·0
	I.C.	1·0	0·9	—	1·1	—	1·0	—	1·1
Fe 25	R.T.	0·9	1·1	1·5	1·8	2·7	3·5	4·5	5·6
	I.C.	1·1	1·1	—	0·9	—	1·0	—	1·0
Cu 2	R.T.	1·0	1·1	1·4	1·7	2·2	2·8	4·0	12·0
	I.C.	1·1	1·4	—	1·7	—	2·2	—	3·0
Cu 10	R.T.	1·0	1·0	1·2	1·3	1·7	2·5	12·0	39·0
	I.C.	1·0	1·6	—	2·0	—	2·6	—	3·4

and another batch in an ice-chest (0–4° C.). Acidity determinations were carried out over a period of 7 days (Table III), and peroxide determinations were carried out daily on the "room temperature" samples, and on alternate days on the "ice-chest" samples (Table IV).

The results given in Tables III and IV are shown in Figs. 2 (acidities), 3 and 4 (peroxide content). A study of the tables and figures reveals that:

(*a*) There was no change in acidity in any of the samples kept in the ice-chest during the observation period of 7 days.

(*b*) The samples kept at room temperature behaved in a parallel manner

to that described for the samples of Table I, the various amounts of different metals depressing lipase activity to approximately the same degree. Ten parts per million of copper inhibited lipase activity completely.

(*c*) Peroxide formation during the first 5 days, *i.e.* during the period required to attain approximately maximum acidity values, varied directly with the content of free acidity, as determined by titration.

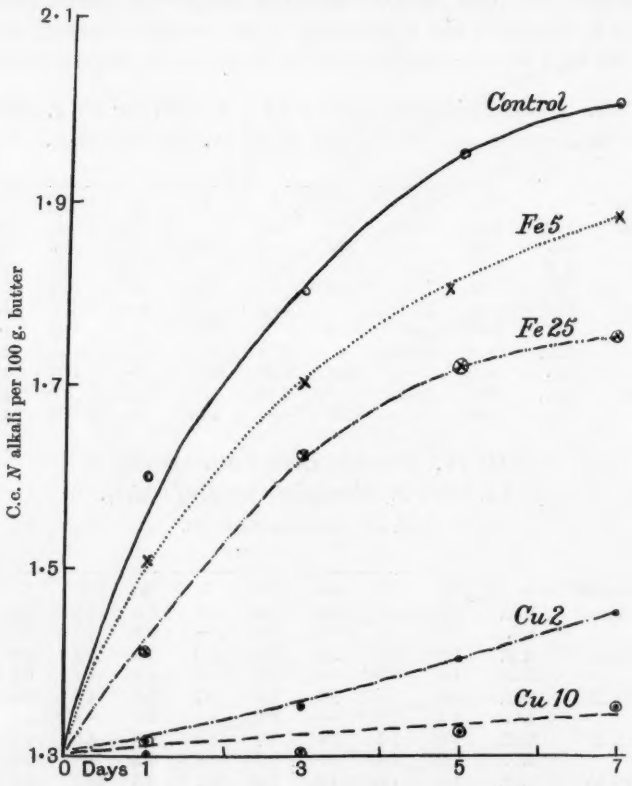

Fig. 2. Unpasteurised sweet cream butter. Development of acidities in the variously treated samples at 15–18° C.

(*d*) The rate of peroxide formation after the fifth day increased directly with the depressing capacity of the various amounts of metals on lipase activity when studied for the first 5 days.

(*e*) The peroxide content of the samples stored in the ice-chest did not increase in the control and the "ironed" samples, but the presence of 2 and 10 parts per million of copper, especially the latter, caused some peroxide formation to occur.

(*f*) The lower temperature of storage and the consequent absence of free

fatty acid liberation prevented the catalytic action of iron in promoting auto-oxidation from taking place during the first 7 days. The catalytic action of copper working under the same acidity conditions at each temperature was considerably decreased by storage at the lower temperature (to about a quarter of its value).

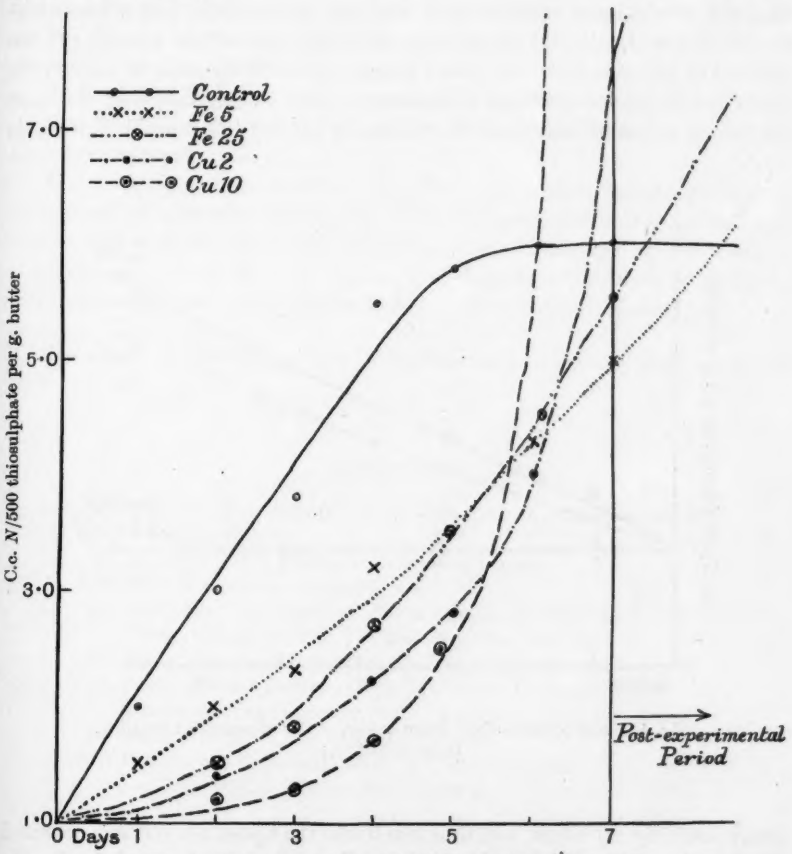

Fig. 3. (Room temperature 15–18° C.) Sweet unpasteurised cream butter. Rates of peroxide formation.

(*g*) During the first 5 days of the life of the butter samples, the traces of metals did not appreciably catalyse oxidation of the fat, the peroxide formation in the liberated oleic acid being more rapid.

DISCUSSION OF RESULTS.

Peroxide formation in butter in which lipase is active is apparently due to the activity of free oleic acid, since (*a*) the amount of peroxide as determined by Lea's method varies directly with the extra acidity formed with time, and

(*b*) where the lipase activity is reduced by traces of various heavy metals, the lower amount of fatty acid liberation is accompanied by a lower amount of peroxide formed. The evidence thus points to oxidation as being partly responsible for the destruction of lipase activity. The attainment of a constant acidity from the fifth day of the butter's life onwards may also be attributed to the inhibition of lipase activity by its products, the free fatty acids. The oxidation of oleic acid, although measured statically by this method according to the amount of peroxide present at given times, is actually dynamic in nature, the peroxides being intermediate compounds. It is conceivable that the part played by traces of heavy metals consists in helping the peroxide formed to

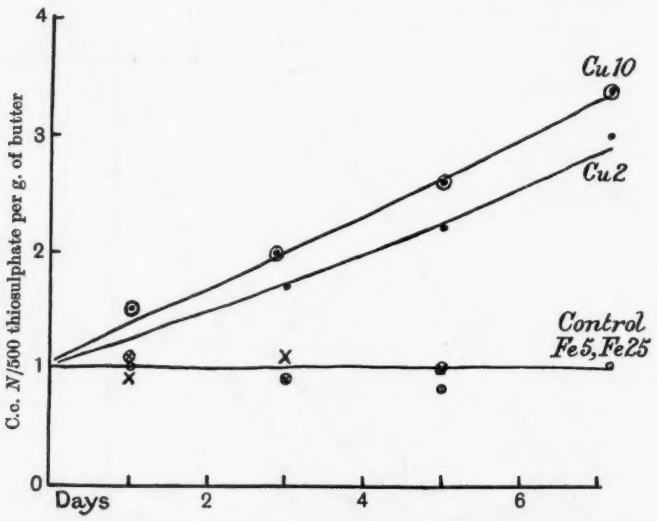

Fig. 4. Sweet unpasteurised cream butter. Rates of peroxide formation. (Kept at 0–4° C.)

break down by oxidation, and thus inactivate the lipase, since it was observed (Table IV and Fig. 3) that the degree of lipase inactivation varied with the subsequent rate of peroxide formation when the fat proper was being oxidised. Traces of various compounds, *e.g.* carotene, fat-soluble vitamins, and lecithin have been proved to play the part of "antioxygens" or "inhibitors" to fat oxidation (Mattill(10, 11)) and are broken down themselves under conditions in which any fat is allowed to oxidise. The traces of lipase in butter considered as a compound plays a similar part in that it is susceptible to breakdown under conditions of low active oxygen concentration, the ease of its breakdown depending on the activity of an activating catalyst with which it may be associated, *e.g.* traces of heavy metals.

SUMMARY.

Traces of heavy metals in amounts up to 25 parts per million have the property of inhibiting the action of lipase in butter from unpasteurised sweet cream in varying degrees depending on the concentration of the metal and on the metal itself. Copper was the most potent metal of those studied, iron, nickel, cobalt, manganese, and chromium being less active, while tin and aluminium had no effect.

The acidity produced was accompanied by fat-peroxide formation in amount varying directly with the acidity. This was attributable to the free oleic acid formed.

The depression of lipase activity by the various metals varied directly with their catalytic power in inducing fat oxidation later, and it is suggested that destruction of lipase was catalysed by the traces of heavy metals according to their varying powers of activating oxygen. Lipase as a compound naturally associated with fats might thus be looked on as a natural "antioxygen."

The author is indebted to Miss D. V. Dearden for the dairy work connected with this investigation.

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39. AN AUXILIARY EVAPORATING AND PREHEATING APPARATUS FOR DRYING WHEY, MILK AND OTHER LIQUIDS BY THE ROLLER PROCESS

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(With 4 Figures.)

The development of the factory-cheese industry in this, and other countries, is handicapped by the wastage of the by-product. In Great Britain alone, approximately 20,000,000 gallons of whey are produced annually by cheese factories(1), and most of it is thrown down the drains. When it is considered that whey, which constitutes half the valuable food material of milk, is so wasted, the development of the industry to even its present dimensions is a matter for surprise.

The farm-cheese industry, which accounts for about three-quarters of the output of cheese in Great Britain, makes better use of its by-product, by feeding pigs and farm stock. It has been shown by experiments at the National Institute for Research in Dairying, and elsewhere, that such valuable foods as milk by-products give the most profitable return when used sparingly, though unfortunately the pig is often used as an alternative to the drain, for the disposal of too large quantities of whey and even separated milk. This is partly due to the irregular supply of dairy by-products which cannot be stored for long. The chief objection to keeping whey beyond the limits of the cheese-making season is its bulk. In order to deal with this difficulty, the late Mr James Ismay, of Iwerne Minster, built a very large tank on the side of a hill in which the summer's supply was made to last over till the next season. Although many changes took place and the whey became a thin black liquid, it still served as a useful addition to the food of pigs.

The expense and difficulties of storing whey, however, indicated the removal of the water as a necessary preliminary. The process of drying also leads to the more profitable utilisation of the product, by supplying markets for its use as a food for human beings or, until such markets are developed, for its more economic use for improving the nutritional value of the cereal foods for pigs.

On May 30, 1922, a grant was made to us from the Royal Agricultural Society of England for "the development of experiments on drying whey and meal as food for pigs." The work under this grant first consisted in exploring the suitability of various improvised tunnel driers for the purpose. Various absorbents were used to carry repeated applications of whey.

From these earlier experiments it appeared that the cost of evaporation

must be reduced if the drying process was to be economically sound, and that an apparatus must be selected of simple design, moderate cost, and easy manipulation, and adapted for use in cheese factories.

The Hatmaker type of twin roller driers appeared, most nearly, to fulfil these requirements.

The principle of roller drying consists of a simple single effect evaporation, the whole process of drying being accomplished by boiling off all water in the liquid and scraping off the dried product that is deposited on the rolls. As the capacity of the rolls is limited by the water content of the product being treated, it is clear that any treatment that preconcentrates or preheats the liquid to be dried will have a direct influence on the capacity of the rolls. Furthermore, most roller driers are designed and adjusted to treat liquids of a given solid content, so that it would be a most desirable feature if some arrangement could be effected whereby a second effect could be applied to any liquid treated and so give a set of roller driers a more extended range in dealing with liquids of variable solid content. The auxiliary apparatus described below has been designed to utilise the evaporative and preheating effect of the thermal units, which have hitherto been wasted in the steam arising from the valley of the drying rolls. This auxiliary considerably increases the efficiency of the rolls.

The results of the earlier experiments with tunnel drying, suggested the possibility of using this principle for the purpose. An oblong chimney of corrugated iron was therefore improvised and fitted over the boiling whey in the valley between the rolls. Whey was run over the outside of this chimney, and the condensed steam on the inside was diverted. The whole apparatus was covered with wood, and a current of air was drawn up over the surface of the whey as it came down over the sheets. From the start the results were encouraging.

At this time Mr J. C. Stead came to the Institute, and joined in the work. He suggested the use of a preheating tank, and in view of the possible future developments of the apparatus a patent(2) was applied for.

In this apparatus a narrow trough, 4 ft. deep, was used as a preheater provided with overflow pipes near the top, which discharged on to the evaporating sheets. The whey passed straight from the bottom edge of the sheets on to the rolls. This apparatus confirmed our expectations as to the improved rate at which whey could be dried, and to the concentration which took place on the sheets. There were, however, two main difficulties to overcome. These concerned the spread of the liquid on the evaporating sheets which was very unsatisfactory, being limited, as it was, to the volume of whey which the rollers could take. Also, the preheating tank was too deep and narrow to admit of satisfactory cleaning. Many investigations had to be made to test the most suitable volume of air which should be passed over the evaporating sheets. In the case of the evaporating sheets, a small scale apparatus was made in which variations in the effect of alterations in the angle and surface could be worked out. Corrugations, such as are used in milk coolers, were found to be

unsuitable on account of the spray which was entrained by the current of air from the projecting corrugations. This confirmed the selection of a smooth tinned copper sheet, hanging in a vertical position, as the best type of evaporating sheet. The next problem was the uniform distribution of liquid on these sheets. Automatic spreading brushes were tried, but the problem was subsequently solved by increasing the volume passing over the sheets and by placing gutters at the bottom, fitted with pipes for diverting the liquid which was in excess of the capacity of the rolls. By this means it has been found possible to circulate as much as 600 gallons per hour through the apparatus, greatly increasing the heat transference thereby. The means of distribution of the whey was also investigated, and after trying various types of feed pipes and spreaders, rounded weirs were found superior to all other types which had been tried. These were fitted both at the top and bottom of the evaporating sheets, and by this means the liquid from the preheater was evenly spread over the sheets in a uniform cascade, and an even distribution from the bottom gutters to the rolls was obtained.

In the course of the work it was found possible to reduce the size of the preheater first used, without loss of efficiency. Easy access for cleaning was a necessity and the final type selected can be cleaned out without removal of screws or end-plates which were features of earlier attempts to retain the dimensions first used, and now proved, by experiment, to be unnecessary.

In principle the apparatus is an evaporative condenser, and as such is designed to utilise all of the available thermal units in the steam arising from the rolls. There are four component processes involved.

Preheating:

I. The heat exchange between the steam arising from the rolls and the cooling surfaces of the condenser and preheater.

II. The metal to liquid exchange on the outside of the sheets and the inside of the preheater trough.

Evaporation:

III. The connective heating by the liquid of the air and its associated vapour in the space between the sheets and the cover.

IV. The process involving the conversion of a portion of the treated liquid into vapour at air temperature.

The first three processes are heat transmission problems and involve a heat transmission coefficient from steam to liquid, air speeds passing the preheated liquid, and the temperature difference.

The fourth process, which involves the evaporation of a part of the water in the liquid being treated, is an essential feature of the method and is influenced by the vapour pressure difference between the liquid and the air.

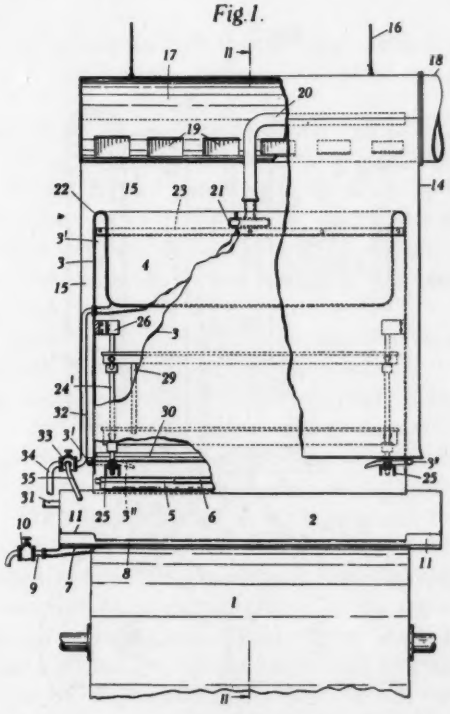
The auxiliary evaporating and preheating apparatus as now used(3) is

illustrated diagrammatically in Fig. 1 (a side elevation), and in Fig. 2 (an end elevation).

In Fig. 1 parts are broken away to show the apparatus in section. Fig. 2 is a vertical cross-section on the line *II–II* of Fig. 1. Fig. 3 is a horizontal cross-section on the line *III–III* of Fig. 2. Fig. 4 is a vertical cross-section through the preheater trough and its associated parts on the line *IV–IV* of Fig. 3.

Referring more particularly to the illustrations in which similar references indicate similar parts, 1 represents the steam-heated drying rolls, the mounting of which is not shown, 2 shows the lower portions of the evaporating sheets (3),

Fig. 1.

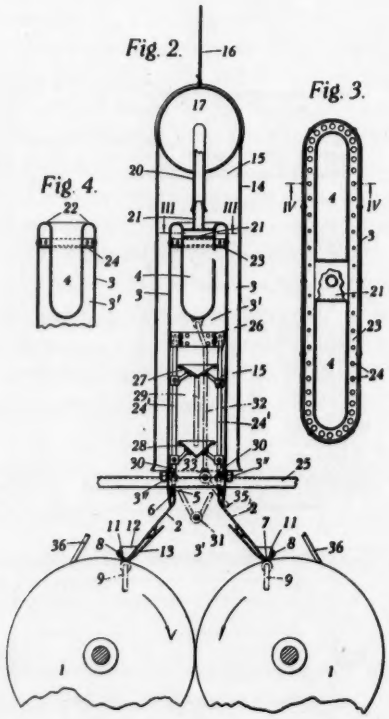


which are shown integral with a preheater trough (4) arranged above the V-shaped space between the rolls(1).

As shown, the lower portions (2) of the evaporating sheets are bent over at the top at 5, to fit into grooves or channels (6), provided near the lower edges of the sheets (3), into which they slide from one end, and by which they are suspended. These lower portions of the evaporating sheets are extended beyond the ends of the rolls and make two sides of a hood-like device through which the steam from the V-shaped space beneath the rolls is drawn into the steam space between the evaporating sheets. The gutters (7) at the bottom of the

evaporating sheets, have the weirs (8) over which the liquid may flow on to the rolls when not drawn off by conduits (9) controlled by means of the taps (10).

The hood (14) forming a vapour space (15) around the evaporating sheets (3) may be counterpoised and suspended by cables (16) so that it can be raised or lowered easily, or it may be arranged to travel endwise upon suitable supports, when, as sometimes happens, head-room is not available in the factory. In such a case the end of the hood shown at 14, Fig. 1, remains fixed and is adapted to make a substantially airtight joint which can be tightened with spring clips.



The hood (14) is provided at the top with an exhaust duct (17), which connects with the duct to the fan, not shown. A slight pull is also exerted through the pipe (20) removably connected with the hollow T-shaped member (21), forming a transverse bridge near the top of the trough (4).

Curved weirs (22) are shown connecting the evaporating sheets (3) with the trough (4), over which the liquid flows in an even cascade on to the sheets.

A baffle plate (23), also shown in Fig. 3, strengthens the weir and distributes the steam over the inner surfaces of the preheater and evaporating sheets.

The trough (4), with the sheets (3), form one unit, which is removably supported on adjustable bearers (24) mounted on suitable beams (25) and brackets (26). Combined gutters and baffles (27 and 28) serve the purpose of collecting condensed water and directing the steam on to the sheets (3). The collection of all the condensed water is an essential function of the apparatus, and other gutters for removing it are shown at 13 and 30. The pipe (31) conducts away all the condensed water above this point. The relatively small quantity collected in 13 discharges over the end of the rolls.

The feed pipe (32) is fitted with a two-way cock (33), through which the preheater (4) may be emptied at the end of the day's work through the side tube (35).

The pipe (34) leads from the bulk storage supply tank, into which also, the excess liquid not required on the rolls (1) is diverted and regulated by the taps (10).

It is estimated that the apparatus should dry half as much liquid again as the rolls alone, or in other words, that when fitted with the evaporative preheater, two sets of rolls should dry as much as three sets not so fitted, and this without increasing the steam consumption on the two sets of rolls.

This anticipation has been realised in a large number of trials. The apparatus has also been used with success for drying milk and separated milk. It might also be used for other liquids.

Another development (4) has resulted in the manufacture of a new product consisting of approximately equal parts of separated milk and whey.

These liquids tend to coagulate if mixed separately before drying, but by feeding the whey over the preheater and adding the milk directly into the valley between the rolls, the dried product comes away from the knives on the rollers in a sheet similar to that obtained when drying milk alone.

Table I gives the results obtained in six typical and recent experiments designed to test the performance of the preheating and pre-evaporating apparatus described above. The experiments were conducted in pairs, one experiment in each case being carried out when the apparatus was in use, with liquid being run over the sheets. These are numbers I, III and V. In the control experiments II and IV, the preheater and evaporator remained in position and the fan was kept running. The sheets in this case acted as a very efficient hood, and by quickly removing the steam from the steam space, improved the rate of dry on the rolls alone. From previous experience the rate of dry on the rolls alone at Shinfield was only about 50 gallons per hour. In the case of experiment VI the preheater and sheets were removed, leaving only the hood and fan.

The experimental runs were, in all cases, of 2 hours' duration, the time of start and finish being taken while the apparatus was running. The average temperatures and pressures were calculated from four half-hourly readings.

In determining the condensate from the rolls, the waste water and steam were passed through a pipe fixed in a tank of cold running water and thereafter

the condensate was passed through a water meter. The figures so obtained were much higher than those obtained in previous experiments where the taps discharged through a long pipe into a tank, and in which the condensed water was measured. The reduced volume in these previous results was probably due to loss of steam.

The first figure for the liquid dried per hour was obtained from the exact measurement of all the liquid before drying. The "calculated" rate of dry serves as a check, and was determined throughout the run from the time taken to dry consecutive 17-gallon churns. This result is subject to considerable error due to frothing, and the difficulty of determining the height of the boiling liquid in the valley between the rolls. It serves, however, as a rough check.

Table I. *Examples of recent results obtained with evaporative preheater and rolls as compared with rolls alone.*

Experiment conducted at ...	Shinfield		Shinfield		Another dairy	
Liquid dried ...	Full cream milk		Whey		Whey	
Experimental reference ...	I	II	III	IV	V	VI
Date (1931) ...	Sept. 1	Sept. 7	Sept. 9	Sept. 10	Nov. 26	Nov. 27
With evaporative preheater ...	Expt.		Expt.		Expt.	
Without evaporative preheater ...		Control		Control		Control
Duration of experimental run (hours)	2	2	2	2	2	2
Total solids in sample (%)	12·47	12·67	6·87	6·89	6·53	6·60
Fat in sample (%)	3·45	3·63	0·25	0·28	—	—
Average temperature of liquid as added to trough (° F.)	58	49	82	80	87·6	84
Average steam pressure on rolls (lb.)	38	38	36·5	39·5	40	45
Atmospheric temperature of room (° F.)	—	59	65	70	54	56
Condensate from rollers (gal. per hour)	83	78	71	69	102	140
Liquid dried (gal. per hour)	88·5	63	80·5	61·5	87·2	58
Calculated from average (gal. per hour)	90	64	79·9	61·7	89·2	—
Temperature in bottom trough passing to rollers (° F.)	173	—	162	—	158	—
Temperature of liquid in top trough (° F.)	133	—	136	—	—	—
Total weight of product dried per hour (lb.)	109¼	80½	55	43·5	58	40
No. of gallons to 1 lb. powder	0·81	0·78	1·45	1·41	1·5	1·49
Moisture in product (%)	4·31	4·80	6·4	7·2	7·29	8·42
Horse-power	7·08	7·08	6·09	6·19	9·3	9·3
Condensed water from steam space per hour (gal.)	21¼	4½	16½	—	30·7	—
Weight of liquid evaporated per hour (lb.)	928·4	650·2	826·7	631·6	898·2	597·4
Weight of water evaporated per hour (lb.)	808	560	766·4	584·9	835·3	554·9
1 lb. steam evaporates lb. water	0·97	0·718	1·08	0·847	0·82	0·396

The horse-power in V and VI was divided as follows: 3·1 H.P. for the fan and pump and 6·2 H.P. for the rolls alone. The total is higher than that at Shinfield, partly because of the lower voltage and higher amps used and partly because 660 gallons per hour were circulated over the preheater by means of a more powerful pump. The use of the larger pump had many advantages over the smaller one in use at Shinfield, which was only capable of 120 gallons per hour.

In both cases a similar Keith Blackman 12-in. fan was used, drawing about 4000 c. ft. per min.

The cleaning of the sheets and preheater present no difficulties when over 100 gallons per hour are used, for no solid milk or whey is then deposited on the sheets. Some solid matter is always deposited on the extension sheets, but as these can be removed and placed in water to soak they can be easily cleaned.

The saving in steam, and consequently in coal, is apparent from the examination of the figures given in Table I. The exact cost can only be worked out in each factory where it is used.

The product is very good and compares well with that obtained by means of the rolls alone. Much co-operative work has been done to open up new markets for the product. The dried whey has been found excellent as an improver in the baking industry, and all the products should find markets as food for human beings.

Experiments have also been conducted which show the value of the dried whey for pig feeding, and the return it gives, when used sparingly, makes it a most valuable food for this purpose.

Our thanks are due to the Royal Agricultural Society of England for the grants with which the earlier part of this work was started, to Lord Iveagh and the Directors of Deco, Ltd., which company has provided funds for its continuance, and also to members of the staff of the National Institute for Research in Dairying, and of the factories of Messrs Aplin and Barrett where the apparatus has also been tested.

The illustrations are taken from Patent Specification No. 360,038, and we wish to thank the Controller of H.M. Stationery Office for permission to use them.

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40. HEREDITY AND MILKING FUNCTION

THE INHERITANCE OF MILK AND BUTTERFAT IN RED POLL CATTLE

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This study is a continuation of the investigations of Prof. Agar, Professor of Zoology, University of Melbourne, entitled "Heredity and milking function," which appeared in the *Journal of Agriculture of Victoria*[1] in January, 1926; and it deals with the inheritance of milk, butterfat percentage, and butterfat in the Victoria Stud of Red Poll Cattle owned by the Department of Agriculture of Victoria, at the State Research Farm, Werribee. The milk and butterfat records used in these investigations are the 273 day records performed under the regulations governing the Victorian Government Pure-bred Herd Test, published annually, and included in the successive volumes of the *Red Poll Herd Book of Australia*. They cover the period since the establishment of the Government Herd Test—1912–13 to 1929–30.

Owing to the fact that a cow's production increases with age up to a certain stage, and then decreases slightly, all records had to be corrected for age, so as to have them on a comparable basis, and these corrected records have been used throughout. To correct a cow's performance for age, correction factors are required. To obtain these factors the production at 6 years old has been regarded as unity, and the corrected records are in terms of production at 6 years old.

Table I sets out the correction factors, together with the average production of 217 cows, varying in age from 2 to 14 years. These 217 cows have 538 records between them.

Table I. *Change in production with age (average for 217 cows).*

	Age in years												
	2	3	4	5	6	7	8	9	10	11	12	13	14
Butterfat (lb.)	262	290	331	335	364	358	365	361	334	343	352	358	294
Correction factor	1·40	1·26	1·11	1·09	1·00	1·02	1·00	1·02	1·10	1·07	1·04	1·02	1·24
Milk (lb.)	6011	6662	7538	7818	8590	8604	8439	8410	8009	7878	7690	8551	7031
Correction factor	1·43	1·29	1·14	1·09	1·00	1·00	1·02	1·02	1·07	1·08	1·12	1·00	1·23
Butterfat percentage	4·40	4·39	4·40	4·26	4·25	4·19	4·31	4·29	4·17	4·36	4·61	4·61	4·18

It will be noticed in Table I that production increases up to the age of 6 years, and then it shows very little variation for the ages of 6, 7, and 8 years. Production then begins to decrease. Owing to the comparatively small number of cows that have been tested at 10 years old or over, the decrease of yield with age is rather irregular, and the records of cows 10 years old or over have,

[1] An abstract of this paper appears on p. 336 of this *Journal*.

therefore, not been considered. The correction factors show close resemblance to those obtained by Prof. Agar who dealt with the records of 98 cows only.

The butterfat percentage or "test" of cows varies only very slightly, and then somewhat irregularly, with age. It is, therefore, generally considered unnecessary to correct butterfat percentage for age. Nevertheless, the writer corrected for age and obtained almost identical results, indicating that the procedure of correction for age is not warranted.

The genetics of farm animals is a very complex study, and modern geneticists still find that the correlation method of studying the inheritance of characters is the simplest. Correlations show the degree of resemblance between parent or offspring or other relatives in respect to certain characters. If the correlation coefficient is 1, then there is perfect resemblance between the groups of individuals under consideration in respect to the character or trait that is being studied, and if the correlation coefficient is 0 then there is no resemblance whatever. When working with large numbers (over 1000) of animals that are existing under similar environmental conditions, the correlation coefficients, between parent and offspring, for such characters as milk yield, butterfat percentage, and butterfat, are approximately 0·5[1]. In this investigation correlation coefficients of approximately 0·35 have been obtained which are regarded as being very satisfactory for three reasons, namely, (1) the moderate number of animals that it has been possible to work with, (2) the breed is a dual purpose breed, and (3) the changes that have occurred in the stud during the 18 years that the cows have been under test.

These changes relate to improved pasturage conditions and to rigid culling which has eliminated those cows which were neither producing highly themselves nor breeding "true to production."

The means (M), standard deviations (σ), and correlation coefficients (r) between mothers and daughters in respect to total milk yield, butterfat percentage, and total butterfat have been worked out and are tabulated in Table II.

Table II. *Means, standard deviations, and correlation coefficients.*

	Milk			Butterfat percentage			Butterfat		
	M	σ		M	σ		M	σ	
	lb.	lb.	r			r	lb.	lb.	r
Mothers	8550	1330	$0{\cdot}321 \pm 0{\cdot}049$	4·38	0·42	$0{\cdot}388 \pm 0{\cdot}046$	368	56	$0{\cdot}316 \pm 0{\cdot}049$
Daughters	8650	1765		4·39	0·40		372	65	

Table III is the correlation surface for 155 pairs of mothers and daughters in respect to butterfat percentage. Having obtained the standard deviations and the correlation coefficient, what is known as the "Regression coefficient" can be worked out. In this case the regression coefficient $= \frac{0{\cdot}388 \times 0{\cdot}40}{0{\cdot}42} = 0{\cdot}370$, and by its use the expected test of the daughter can be arrived at, when the

[1] Refer to *Milk Secretion*, by J. W. Gowen. Baltimore: Williams and Wilkins Co., 1924.

test of the mother is known. The daughters on the average will have 0·370 time the deviation of their mothers. If a mother has a test of 4·80, then she has a deviation of 0·42 point from the mean of all others which is 4·38. Now the expected deviation in test for a daughter of such a mother will be

$$0{\cdot}370 \times 0{\cdot}42 = 0{\cdot}16$$

point from the mean of all daughters which is 4·39. Therefore the expected test of the daughter will be $4{\cdot}39 + 0{\cdot}16 = 4{\cdot}55$. By similar calculation the expected test of daughters of other mothers may be arrived at.

Table III. *Correlation surface of butterfat percentage of mothers with that of daughters.*

Daughters %	Mothers 3·5–3·6	3·6–3·7	3·7–3·8	3·8–3·9	3·9–4·0	4·0–5·1	4·1–4·2	4·2–4·3	4·3–4·4	4·4–4·5	4·5–4·6	4·6–4·7	4·7–4·8	4·8–4·9	4·9–5·0	5·0–5·1	5·1–5·2	5·2–5·3	5·3–5·4	5·4–5·5	5·5–5·6	
3·5–3·6	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
3·6–3·7	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
3·7–3·8	.	.	.	.	.	3	.	2	1	.	.	.	.	.	.	.	.	.	.	.	.	6
3·8–3·9	1	.	.	.	.	1	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	4
3·9–4·0	.	.	.	.	1	1	2	.	1	1	1	.	1	.	.	.	.	.	.	.	.	8
4·0–4·1	.	.	1	1	.	1	2	1	2	.	.	1	.	1	.	.	.	.	.	.	.	10
4·1–4·2	.	.	.	1	.	1	4	3	2	1	.	3	1	1	.	.	.	.	.	.	.	17
4·2–4·3	1	.	.	.	.	2	1	2	.	2	1	1	1	1	.	.	.	1	.	.	1	14
4·3–4·4	.	.	2	1	.	3	2	3	4	.	2	1	.	.	.	.	.	.	1	.	1	20
4·4–4·5	.	.	.	.	.	1	2	2	2	1	2	.	1	1	.	.	.	.	.	.	.	12
4·5–4·6	.	.	.	2	.	3	1	2	2	1	1	.	6	1	.	.	.	.	.	.	.	19
4·6–4·7	.	.	.	.	.	.	1	1	1	.	1	1	1	1	.	.	.	.	.	.	.	7
4·7–4·8	.	.	1	1	1	2	.	1	1	3	1	.	.	.	.	.	.	.	.	.	.	11
4·8–4·9	.	.	.	.	.	.	.	.	.	.	1	.	1	2	.	.	.	.	.	.	2	6
4·9–5·0	.	.	.	.	.	1	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	3
5·0–5·1	.	.	1	.	.	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	3
5·1–5·2	.	.	.	.	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	2
5·2–5·3	.	.	.	.	.	.	.	.	2	.	.	.	.	1	.	.	.	2	.	.	.	5
5·3–5·4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	2
5·4–5·5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5·5–5·6	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
	4	.	6	6	3	19	16	18	20	11	11	7	13	10	1	.	.	3	1	.	6	155

Mothers testing above the average are expected to have daughters testing above the average, and this will be the case if a number of daughters are considered, although individual daughters may vary considerably; but daughters generally will only be 0·370 time as much above the average of all daughters, as the mothers are above the average of all mothers; and similarly mothers testing below the average will have daughters testing below the average, but only 0·370 time as far below the average of all daughters, as the mothers are below the average of all mothers.

It is thus seen that a daughter does inherit some of the factors that contribute in the formation of the character known as butterfat percentage or test from its mother or dam. The remainder of these factors are inherited from the sire and other ancestors who have transmitted these factors, as recessive factors through the parents, to the offspring where they are now appearing.

Tables IV and V are the correlation surfaces for total milk yield and total butterfat respectively between 155 pairs of mothers and daughters.

Total milk yield and butterfat are influenced more by environmental conditions as distinguished from hereditary factors than is butterfat percentage;

Table IV. *Correlation surface of total milk yield of mothers with that of daughters.*

Daughters lb.	Mothers 5,000	5,500	6,000	6,500	7,000	7,500	8,000	8,500	9,000	9,500	10,000	10,500	11,000	11,500	12,000	12,500	13,000	13,500	
5,000	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	2
5,500	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	2
6,000	.	.	1	1	1	.	.	1	.	.	.	.	.	.	.	.	.	.	4
6,500	.	.	.	1	1	1	3	1	1	1	.	.	.	.	.	.	.	.	9
7,000	.	.	.	1	.	.	2	1	3	.	2	.	.	.	.	.	.	.	9
7,500	.	.	.	1	1	4	3	4	1	.	2	.	1	.	.	.	.	.	17
8,000	1	1	.	1	2	5	1	1	4	.	3	.	.	2	1	.	.	.	22
8,500	.	.	.	.	3	4	2	3	2	1	1	.	.	.	.	.	.	.	16
9,000	.	.	2	1	1	1	7	7	3	3	.	.	.	.	1	.	.	.	26
9,500	.	.	.	2	.	2	2	5	1	.	2	.	.	1	.	.	.	1	16
10,000	.	.	.	.	2	1	2	2	.	.	2	.	.	.	1	.	.	.	10
10,500	.	.	.	.	.	.	.	1	.	1	1	.	.	.	1	.	.	.	4
11,000	.	.	.	.	.	.	1	1	2	1	1	.	.	.	.	.	.	.	6
11,500	.	.	.	.	.	.	1	1	1	2	.	.	.	.	.	.	.	.	5
12,000	.	.	.	.	.	.	.	.	1	1	1	.	1	1	.	.	.	1	6
12,500	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13,000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13,500	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
	1	1	3	9	11	18	24	30	20	10	15	1	2	4	4	.	.	2	155

Table V. *The correlation surface of total butterfat of mothers with that of daughters.*

Daughters lb.	Mothers 225	250	275	300	325	350	375	400	425	450	475	500	525	550	
225	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
250	.	.	.	.	1	2	.	.	.	.	.	.	.	.	3
275	.	.	1	2	4	1	4	2	.	.	1	.	.	.	15
300	.	1	1	1	.	5	1	.	.	1	.	1	.	.	11
325	.	.	2	.	5	2	3	.	1	.	2	.	.	1	16
350	.	1	.	6	3	6	6	2	3	.	.	.	.	.	27
375	.	.	1	2	4	8	2	.	2	2	.	.	.	.	21
400	.	.	2	2	2	5	6	1	2	.	1	1	.	.	22
425	.	.	.	.	1	2	4	.	.	2	1	.	.	.	10
450	.	.	.	.	1	.	4	1	2	.	1	1	.	.	10
475	.	.	.	.	1	1	4	1	3	1	.	.	.	.	11
500	.	.	.	.	1	.	1	1	1	3	1	.	.	.	8
525	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	.	2	8	13	23	32	35	8	14	9	7	3	.	1	155

and greater fluctuations with less correlation between mothers and daughters may be expected in these respects.

It will be noticed that the correlation coefficients for both milk yield and butterfat between mothers and daughters are lower than is the correlation coefficient for butterfat percentage.

The correlation coefficients for milk yield, butterfat and butterfat percentage are all greater now than when Prof. Agar made the original investigation on the "Victoria" herd. The variability in respect to milk yield and total butterfat is greater in the case of mothers than in the case of daughters: whereas in respect to butterfat percentage the daughters are the more variable.

The influence of the bull.

By the use of the results set out in the preceding tables it is possible to determine which bulls are increasing production in the herd, and which are not, and in Table VI the performance of the bulls used in the herd under consideration are tabulated.

Table VI. *Influence of the sire on daughters' production.*

	Milk					Test—butterfat percentage				Butterfat			
	Number of daughters				Mean difference of daughters from expectation (lb.)	Number of daughters			Mean difference of daughters from expectation (%)	Number of daughters			Mean difference of daughters from expectation (lb.)
Sire	Total	Above expectation	Equal to expectation	Below expectation		Above expectation	Equal to expectation	Below expectation		Above expectation	Equal to expectation	Below expectation	
Nicotine	38	21	9	8	+ 590	4	19	15	−0·16	11	19	8	+ 9
Belligerent	38	16	7	15	− 10	16	14	8	+0·11	11	16	11	+ 4
Longford Major	17	6	2	9	− 120	1	5	11	−0·30	3	5	9	−25
Vice Regal	16	7	1	8	+ 160	4	6	6	−0·06	7	5	4	+ 5
Victoria Bit o' Bacca	15	4	1	10	−1010	6	7	2	+0·23	3	4	8	−18
Tabacum	9	—	1	8	−1770	8	1	—	+0·46	1	3	5	−44
Earl of Beaconsfield	4	—	1	3	− 970	8	1	—	+0·42	—	4	—	0
Victoria Volcano	4	3	1	—	+1340	—	3	1	−0·16	3	1	—	+47
Victoria Air Marshall	2	2	—	—	+1470	1	1	—	+0·28	2	—	—	+87
Other bulls (9)	12	8	2	2	+ 700	1	7	4	−0·16	4	6	2	+10

Cows are counted as "equal to expectation" when they differ from expectation by less than 500 lb. of milk, 30 lb. of butterfat, and 20 points in test.

The case of Belligerent may be cited to explain this table. He has 38 tested daughters from tested mothers, and 16 of these have produced more milk than was expected of them after allowing for the influence of the mother, 7 have produced as much milk as was expected, while 15 have produced less milk than was expected of them. The average production of these 38 daughters was 10 lb. less milk than was expected; and similarly, the columns for butterfat percentage and butterfat can be explained.

Summarising the performance of these bulls, it is found that:

Nicotine raised both milk and butterfat, but the milk more than the butterfat, hence he lowered the test (butterfat percentage).

Belligerent lowered the milk slightly, and raised the butterfat, hence he raised the test.

Longford Major lowered both milk and butterfat, but the milk less than the butterfat, hence he lowered the test,

Vice Regal raised both milk and butterfat, but the milk slightly more than the butterfat, hence he lowered the test slightly.

Victoria Bit o' Bacca has lowered both milk yield and butterfat, but the milk more than the butterfat, hence he raised the test.

Tabacum lowered both milk and butterfat, but the milk more than the butterfat, hence he raised the test.

Earl of Beaconsfield[1] has lowered the milk and left the butterfat unaltered, hence he has raised the test.

Victoria Volcano[1] has raised both milk and butterfat, but the milk more than the butterfat, hence he has lowered the test.

Victoria Air Marshall[1] has raised both milk and butterfat, but milk less than the butterfat, hence he has raised the test.

THE INFLUENCE OF THE GRANDMOTHERS.

No significant correlation for either milk yield, total butterfat or butterfat percentage has been found to exist between the granddaughters and the paternal or maternal grandmothers, due partly to the changing environment, and partly to the heterozygosity of the older members of the stud. Gowen, working with the Friesian Cattle in the United States of America, has found a correlation coefficient of 0·25 for milk and butterfat percentage between grandmothers and granddaughters.

Most studies on the inheritance of the milking function have been carried out on the purely dairy breeds of cattle, such as Friesian, Jersey, Red Danish, and Ayrshire, and in most cases larger correlation coefficients have been obtained than those in the Red Poll herd under investigation. This was to be expected because Red Poll cattle are essentially a dual purpose breed, and the breeder has to select and breed for meat production as well as milk and butterfat production.

THE METHODS OF BREEDING.

The data available makes possible a brief study of the various methods of breeding that have been adopted in this stud herd since its foundation. Before presenting such study, however, a short summary of the different methods of breeding customarily adopted by breeders will be advantageous. These methods are illustrated thus:

1. Cross-breeding.
2. Grading up.
3. Pure breeding
   - (*a*) Outcrossing
   - (*b*) Inbreeding
     - (i) Line breeding
     - (ii) Close breeding.

Cross-breeding is the mating together of two animals (or plants) belonging to different genera, species, breeds (or varieties). The progeny are known as hybrids or cross-breds, and are noted for their vigour, hardiness, disease resistance, and rapid growth. This phenomenon is known as heterosis. The

[1] These bulls have so few tested daughters that these conclusions are subject to variations when more daughters are tested.

mule is a well-known example, and is suitable for hard conditions. It can do more work on less feed than either of its parents (the jack—male of the ass family, and the mare—female of the horse family).

In the meat breeds of cattle, swine, sheep, and even poultry the crossing of distinct breeds is a very common practice. The increased size of the progeny and the rapidity with which the size is obtained, are so outstanding that the method tends to replace pure breeding as far as meat production is concerned.

There is very little scientific data relating to the cross-breeding of dairy cattle in Victoria, but the first generation cross between the Friesian and the Jersey is fairly common. It has the combined dairy characteristics, and is in eager demand at dairy cattle sales.

Grading up is the stepping stone to pure breeding, and comprises the practice of using a pure-bred bull or sire on ordinary grade females. If pure-bred sires are continually mated to the progeny the practice is to be highly commended. The successive progeny will become uniform in appearance, and if the sire is selected judiciously, their production will be increased. After five or six generations the descendants will be to all intents and purposes pure-breds.

All pure-bred animals have been graded up either by natural selection or by man from the original native stock. Some of the best families in the Red Poll Stud under consideration have been graded up from cows of unknown breeding.

Pure breeding is the mating together of animals within a fixed breed.

(*a*) Out-crossing is pure breeding with animals that are unrelated or that show no relationship for the first six generations back in their pedigrees. It is the most common practice in pure breeding, but it is usually accompanied with success only when the breeder luckily selects a sire that will "nick" with his female stock.

It is really a "hit or miss" system, and when practised generally results in the stud remaining stationary or deteriorating.

(*b*) Inbreeding is pure breeding with related animals. Inbreeding is being adopted more and more, and it leads to an accentuation of characters whether desirable or undesirable. When inbreeding is practised severe culling has to be adopted during the first few generations while the undesirable characters continue in evidence and require to be eliminated. Ultimately by this method a homogeneous stud or breed is obtained (*e.g.* the Jersey and Guernsey cattle that have been inbred for two centuries on their respective islands without the addition of any outside blood whatsoever). Intensive inbreeding may lead to loss of vigour and vitality and this has to be guarded against. Inbred animals, when out-crossed with other strains, usually produce progeny with a predominance of the accentuated desirable characteristics.

As stated above, inbreeding may be divided into:

(i) Line breeding, which is inbreeding with more or less distantly but distinctly related animals.

(ii) Close breeding. Close breeding is inbreeding with closely related

animals, such as parent with progeny, full or half sister with full or half brother, etc.

These three methods of pure breeding have been practised in the herd under mention, and Table VII illustrates the results obtained from the three different methods. This table is explained similarly to Table VI.

A synopsis of this table reveals the following results:

(1) The test or butterfat percentage is not changed by the method of breeding.

(2) Out-crossing has slightly lowered both milk yield and total butterfat.

(3) Line breeding has considerably increased both milk and butterfat yields.

(4) Close breeding has materially lowered both milk and butterfat yields.

Line breeding has thus proved to be the most successful breeding method that has been adopted.

Table VII. *Influence of the method of breeding on daughters' production.*

	Milk					Test—butterfat percentage				Butterfat			
	Number of daughters				Mean difference of daughters from expectation (lb)	Number of daughters			Mean difference of daughters from expectation (%)	Number of daughters			Mean difference of daughters from expectation (lb.)
Method of breeding of daughters	Total	Above expectation	Equal to expectation	Below expectation		Above expectation	Equal to expectation	Below expectation		Above expectation	Equal to expectation	Below expectation	
Out-crossing	102	39	19	44	− 92	32	38	32	−0·01	24	45	33	− 4
Line breeding	29	20	4	5	+813	7	13	8	+0·02	16	9	4	+35
Close breeding	24	9	—	15	−425	4	13	7	−0·03	6	8	10	−21

A brief description of the methods of close breeding as applied in this herd is worthy of note. One instance is exemplified by a cross with Victoria Bit o' Bacca (sire, Nicotine) on daughters of Vice Regal (sire, Nicotine) whose mothers were sired by Nicotine. To make this more clear, such a pedigree is elaborated below:

- Daughter
  - Victoria Bit o' Bacca
    - Nicotine
    - Nictitana
      - Nicotine
      - Birdseye
  - Mother
    - Vice Regal
      - Nicotine
      - Velveteen
    - Grandmother
      - Nicotine
      - Great-grandmother

Six daughters thus bred have been tested, and no less than five of them have produced less milk and butterfat than was expected after making the allowance for the influence of the mothers. These cows are also smaller in size than the average of the herd.

Eleven daughters whose parents were half-brothers and sisters, but with no other blood relationship, have production records, and, of these, five have

produced less milk and only two less butterfat than was expected, while the records of one daughter whose parents were full brother and sister, and three daughters whose parents were half brothers and sisters and who also had other blood relationship, show that they have all produced less milk and butterfat than was expected.

The Red Poll cattle in this stud are still showing heterozygosity (genetic variation) as far as milk yield, butterfat percentage, and butterfat are concerned; but nevertheless they are rapidly becoming more and more homozygous (uniform genetically) from year to year.

In conclusion, it may be stated that line breeding and systematic culling of all undesirables, are the methods that have to be adopted before a breed or stud will become fixed at a high level of performance.

This investigation has shown that out-crossing gives almost neutral results, but it should be mentioned that most of the out-crossing was done in the earlier history of the stud, before the present high level was obtained, and that out-crossing now would probably lower the performance of the stud considerably. As the better families were segregated out, inbreeding became the more common practice, and the present herd of 115 females (all ages, August, 1930) contains 28 out-crossed, 64 line bred, and 23 closely bred females.

Most of the inbreeding has been done with Nicotine blood, a fair number of cows have been inbred to Longford Major, but very few have been inbred to Belligerent.

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41. BREEDING FOR MILK PRODUCTION IN THE TROPICS

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(With 1 Plate and 2 Figures.)

Introduction.

A FRESH milk supply is as essential in the tropics as it is in the more temperate regions of the world. The most fundamental of the many problems connected with tropical dairying is that concerned with the establishment of breeds of dairy cattle which will live and thrive in their environment, and produce milk; the intensity of the problem varies with the degree of severity of the climatic and environmental conditions. The manner in which this problem has been dealt with at the Government Stock Farms at Kingston, Jamaica, together with the results, is discussed in the following study. The data were collected by Mr J. Hammond from the records of the herds at these farms. To Mr H. H. Cousins, the Director of Agriculture, who has not only built up the herd to its present state of excellence but has kept also a complete record of the pedigrees and performances of all the animals, and to Dr S. Lockett, Veterinary Officer for the Department, our thanks are due for having put these records at our disposal and for communicating to us the results of their experience.

There are certain features common to the few experiments upon the breeding of dairy cattle for tropical and sub-tropical regions already on record. The introduction of European breeds into the tropics with a view to their final acclimatisation to their new environment is a questionable proceedure. The adaptability of a breed to its surroundings may be measured by its capacity to be self-sufficient as a breeding unit, capable of existing and multiplying without recourse to repeated importations of new blood. When judged by this criterion, the experience of European breeds in the tropics may be said to be one of failure. The first imported animals may be satisfactory, but subsequent generations bred in the tropics show progressive degeneration brought about by the debilitating ravages of disease, the effects of high temperatures, exposure to strong sunlight and an inability to maintain the constitution necessary for their inherent high-milking capacities.

The development, by selection, of milking strains within some of the pure breeds of Indian cattle of the Zebu or Brahmin type has been advocated. Kothavala (6) gives a résumé of the various Indian breeds available, together with their economic characteristics, and is of the opinion that in the proper

handling of the material there available lies the solution of the problem of breeding dairy cattle for tropical countries like India. He records individual yields of 1000 gallons of milk and annual herd averages of 500 gallons already achieved by animals selected within these breeds. Such yields are the exception, however, and Matson (8) puts the general breed averages at a much lower level. In districts with no well-established type, an attempt may be made to improve the native cattle by selection. These indigenous animals are, however, usually extremely heterogeneous, and improvement by this method is liable to be slow and unsatisfactory. This was found to be the case by Cousins (1) in Jamaica, by whom the method was tried and discarded. The Indian breeds naturally possess the constitution to thrive in their environment, one to which they have been exposed for centuries. But the formation of a new breed of dairy cattle is a process which is likely to be protracted. It has taken two centuries to bring the European breeds to their present state of production.

Here are two types of cattle selected for many years for two distinct characters—an Indian type, by natural selection, for a constitution suited to the tropics, and a European type, by artificial selection, for high milk yield. Such comprehensive characters (constitution and milk yield) are undoubtedly the expressions of multiple factor inheritances, and it does seem possible that a scientific blending of the two strains might achieve the desired aim of a new breed combining some of the characters of both parent breeds. The possibilities of the crossing of the two types are, in fact, widely recognised. In southern Texas, 60 per cent. of the range bulls are high grade Zebus, their value in crossing for meat with the European beef breeds being well known. Lush (7), in dealing with an aspect of this practice, provides a justification for the "percentage" or "amount of blood" terminology in the description of individuals as well as of groups in such interbreed crosses, based upon the consideration of the large number of factors involved and the consequent lack of sharply defined segregation. Jensen (4), in describing the position of dairying in Kenya, records the improvement brought about in breeding for milk production by the crossing of the native cattle (Zebu) with European cattle, notably Friesian. Matson (8) gives an account of extensive cross-breeding experiments conducted at the Indian Army Dairy Farms, and discusses the successes of the various crosses and back-crosses tried. The first crosses (half Zebu) gave, in most cases, very satisfactory results. Back-crosses to the European parent, *i.e.* progeny with one-quarter Zebu blood, were equally satisfactory, but a further dilution of the Zebu blood to one-eighth caused much reduced milk yields because of the reappearance of limiting defects commonly affecting the pure European breeds in the tropics. An account of the methods of improving dairy cattle in Trinidad, where crosses of Friesian and Zebu breeds are being experimented with, is given by Metivier (9). The superior yields of the animals three-quarters bred to the Friesian parent when compared with the half-bred are remarked upon, as are also the earlier maturity and greater uniformity of that cross. Efforts to improve dairy cattle in the

French colony of Tunis with native cattle, Zebu and French dairy breeds are described by Ducloux (2). The opinion is held that, for North Africa, 40 per cent. of Zebu blood in the cross is essential, if disease resistance and milking propensities are to be combined in the one animal. Hammond (3) reminds us that promiscuous cross-breeding leads nowhere. He advises a definite plan of procedure in all future cross-breeding work and is of the opinion that adherence to one breed and its crosses is distinctly to be preferred to mixed crossing with several breeds which prevents concerted action along any one line and is liable to lead only to a race of mongrels.

MATERIAL.

The materials for this study consist of the milk records and pedigrees of a dairy herd which has been built up at the Government Stock Farms at Hope, Kingston, Jamaica, by Mr H. H. Cousins, Director of Agriculture. In an endeavour to breed dairy cattle suited to the Jamaican climate, numerous crossing experiments have been tried with the native cattle and imported European and Indian (Zebu) breeds. Very full records of the performances of these animals have been kept, and it is with the treatment of their milk records and pedigrees that this investigation is concerned. The limitations of such a paper study are recognised, it being obvious that many important factors may be disregarded and unequally valued. For example, a certain bull may be responsible for a very valuable infusion of "constitution" into the herd by his daughters, which will be of lasting benefit although not always reflected in the immediate milk records; then, too, the actual inheritance for milk production which an animal possesses may not be fully expressed in the record on account of limitations in feeding—the concentrates necessary for the higher yields not always being available. But in the comparisons of the milk yields of the crosses which follow, it has been assumed that, on the average, when one animal produces a better record than another in the same herd, it is because she possesses more of the hereditary factors to do so, and the constitution to express that inheritance. The milk record data include total lactation yields (computed by daily weighing), with particulars of the month of calving, the length of the service period (the period elapsing between a calving and the next fertile service), and the dry or rest period preceding the lactation.

Standardising the milk records.

In view of the findings of several workers (5, 10), on the effect of these factors upon a lactation yield irrespective of heredity, it was decided to measure their influence in the present data with a view to eliminating some of their effects before studying the inheritance problem. The course followed was similar to that of Sanders (10) in his work on the elimination of variations caused

by these factors on records of cows in the eastern counties in England. The following is a summary of the number of lactations available for the purpose.

Breed	No. of lactations
Native (including Zebu grades)	258
Jersey	63
Red Poll	48
Guernsey	10
Ayrshire	8
Brown Swiss	2
	389

Month of calving. It was found that there were time of year variations in the milk yields, but their size did not warrant the use of a factor to correct for them. The number of lactations available being small, the occurrence of two or three high producers in one month was likely to be reflected in a higher average yield for cows calving in that month. The results are given however, (Table I), because the yields of cows calving in the dry season (December-March) are, on the whole, higher than those calving in the wet season (May-October). Further investigation on this point will be necessary under tropical conditions before it can be taken as proved.

Table I. *The influence of month of calving on yield of milk.*

Month of calving	No. of lactations	Average yields (lb.)	Deviation from annual average	% correction to annual average
Jan.	25	6590	+580	− 8·8
Feb.	32	6181	+171	− 2·8
March	37	6700	+690	−10·2
April	44	6432	+422	− 6·6
May	27	5724	−286	+ 5·0
June	53	5657	−353	+ 6·2
July	29	5865	−145	+ 2·5
Aug.	29	5228	−782	+15·0
Sept.	29	5817	−193	+ 3·3
Oct.	31	5678	−332	+ 5·8
Nov.	28	6197	+187	− 3·0
Dec.	25	6046	+ 36	− 0·6
Average		6010		

Dry period. Of the 389 lactations, 133 are those of first calvers, the large number of them in proportion to those of older cows being characteristic of the failures which occur after the strain of a lactation when there is not the constitution to bear it. The remaining dry periods are too few to work out a dry period correction factor.

Service period. The influence of this factor was studied in two separate sections, first calvers in one lot and cows other than these in the other, treatment which was shown by Sanders to be justified, and capable of explanation on the basis of the different nature of the heifer lactation curve with its long even yield compared with that of the older cows which give relatively more milk in the earlier part of the lactation. Yields according to the various service periods were plotted on graphs and a standard service period of 170 days being chosen in each case, correction factors to it were calculated. The greater length

of service period in the tropics is worthy of comment. The average S.P. for 58 Jersey lactations was found to be 196 days and of 239 "Native" lactations (including Zebu crosses) 222·5 days. The irregularity and lateness in breeding of the Indian breeds is remarked upon by Kothavala. Table II gives a summary of the influence of service period, together with the correction factors evolved.

Table II. *The influence of the length of service period on the yield of milk.*

1st calvers			Others			Service period corrections		
Service period (days)	No. of lacta-tions	Average milk yield (lb.)	Service period (days)	No. of lacta-tions	Average milk yield (lb.)	Interval (days)	First calvers	Others
						0–29	+33·1	+59·8
40–99	9	3785	20–59	15	3769	30–59	+25·0	+40·9
100–139	18	3470	60–79	12	4203	60–89	+17·8	+26·6
140–179	14	4213	80–99	22	4054	90–119	+11·4	+16·2
180–219	15	4218	100–119	23	3819	120–149	+ 5·7	+ 8·6
220–239	14	4358	120–139	22	5132	150–179	+ 0·5	+ 1·0
240–279	11	4380	140–159	16	5127	180–209	− 4·2	− 4·8
280–319	12	5170	160–179	15	4995	210–239	− 8·5	− 9·1
320–399	8	5835	180–219	17	4723	240–269	−12·4	−13·0
400–499	8	7055	220–239	18	5481	270–299	−16·0	−16·3
—	—	—	240–259	12	5611	300–329	−19·3	−18·7
—	—	—	260–299	12	6115	330–359	−22·3	−20·6
—	—	—	300–339	13	6252	360–389	−25·2	−22·2
—	—	—	340–379	16	6140	390–419	−27·8	−23·1
—	—	—	380–419	12	6572	420–449	−30·3	−24·0

Age. With lactation yields corrected for the influence of service period, the influence of age was studied, age in this case being stated in terms of lactations. Possibly by reason of the fact that in the tropics a longer period of time is required for growth and development, it was found that heifers were not calved down until about $3\frac{1}{2}$–4 years old, an unproductive period the length of which, for economic reasons, it would be desirable to shorten, if it could be done without affecting the development of the animal. For the analysis of the effect of age paired lactation comparisons were made—the average yield of first calvers being compared with the average yield of the same group of animals in their second lactation, and so on. A graph (Fig. 1) was plotted of the percentage differences in yield with age and it was found that maximum production occurred at the fifth lactation. From the graph correction factors to this mature standard were deduced. These are as follows:

Correction factors for milk yield to a mature basis.

Age in lactations	Correction factor to maturity (%)
1	+44·4
2	+22·0
3	+10·0
4	+ 3·8
5	0·0
6	+ 2·0
7	+ 6·0

By comparison with the correction factors evolved by Sanders (10) for cows in

England, it will be seen that those found necessary here for age are very similar. It may be that this is so generally. On Fig. 1 were also plotted the average lumped yields of all cows unpaired. The increasing divergence of the two curves is indicative of negative selection for milk, as the upper one, from the paired lactations, shows the physiological trend of the lactation curve with age, and the lower one shows the curve actually obtained from production at the various ages. This can be caused by selection on maximum daily yield in preference to lactation yield ((11), p. 154), but in this case is rather due to

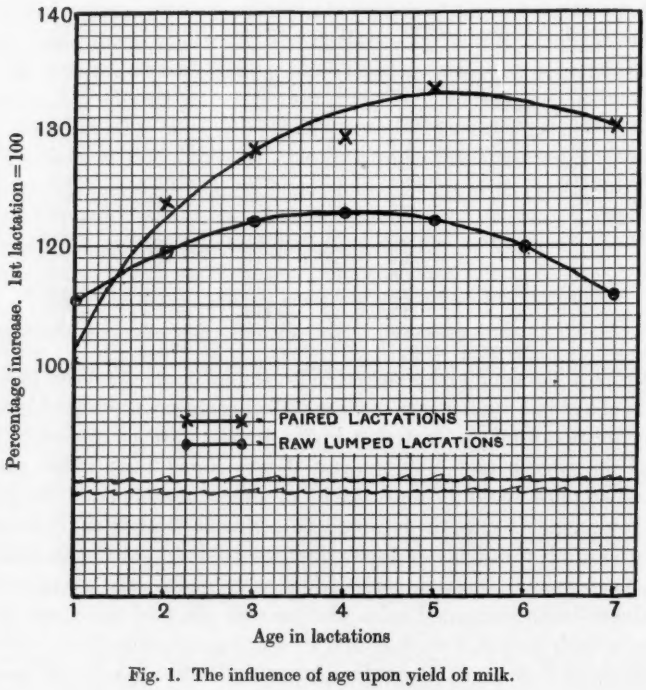

Fig. 1. The influence of age upon yield of milk.

the fact that many of the high-yielding cows have much European blood and have not the constitution for withstanding more than one or two lactations at their normal rate of production.

In evaluating the milk records of cows in the breeding experiments following, it was decided to correct the actual lactation record for age and service period only, on the basis of the above results; for, apart from feeding, these appear to be the most important environmental factors affecting yield. Since all the animals were kept in one herd under similar conditions, the factor of feed does not assume the importance which it does when records of animals subject to different systems of management are being considered.

The breeding experiments.

When, in 1910, a determined attempt to increase the amount of the milk supply of the island was begun, a survey of the material available for such improvement offered several possibilities. The native cattle on the island—mixtures of successive importations of Spanish, Central American, Barbados, Porto Rican, Indian, and Channel Islands stock, had as their main commendable feature hardiness to withstand their environment. Improvement from this stock by selection alone was attempted, but it was observed that such progress would be too slow (1). A breeding policy to secure a constant milk supply was then initiated, based upon the introduction of European dairy breeds and the grading up of native stock to them. Six bulls, two Holstein-Friesian, one Jersey, one Guernsey, one Zebu and a Zebu-Jersey cross have been used with varying degrees of success. It soon became obvious that the pure European breeds could not themselves weather their new environment, degeneration in successive generations being acute owing to the lowering of vitality by disease, high temperature and strong sunlight, and short supplies of suitable nutriment, principally of high protein, and concentrated foods. The nature of this loss in constitution can be illustrated by photographs of the animals. The two Jersey cows (Pl. I, figs. 1 and 2), are representative of the first and second generation bred in Jamaica. Similar changes resulting in lack of constitution followed the grading up of native or Zebu stock with some crosses of European blood. At first the grades showed distinct improvement, but when the percentage of European blood reached a high level this type of degeneration occurred. Pl. I, fig. 3, shows the same marked degeneration in a high grade Jersey cow. The nature of the increase in constitution brought about by the introduction of Zebu blood is seen in comparing this cow with her daughter (Pl. I, fig. 4) by a Zebu bull. What can be done by this combination of the constitution of the Zebu with the udder development of the European is illustrated by Pl. I, fig. 5, Norbrook II, the highest-yielding cow on the island. She has a "mature" record of 16,700 lb. of milk (actual lactation of 18,030 lb. with a maximum daily yield of 66 lb.). She is one-quarter Zebu and three-quarters European with Holstein blood predominating. Pl. I, fig. 6, is an example of an animal with sufficient constitution but lacking in milk-inheritance, possessing, as she does, three-quarters Zebu blood.

In dealing with the results of this investigation, the performance of six bulls will first be analysed (Section A) on the basis of the yields of their daughters as a whole and (Section B) on the basis of the "percentage of blood"—the milk yields of the daughters and dams being compared according to the amounts of Zebu blood in their make-up. In all cases milk-records are calculated to maturity, being corrected for Age and Service Period.

A. *Analysis of bull's progeny.*

Bull No. 1. Experiment. This bull was by a Jersey sire out of a Zebu-Jersey Grade cow. He possessed one-eighth of Zebu blood. The following statement is a summary of his influence on his daughters' milk yields:

Item		Milk (lb.)
Mature average yields of 20 daughters	=	4625
Mature average yields of 20 dams	=	4975
Difference: decrease	=	−350

The decrease in milk yield is not significantly large and the constitution which he was responsible for introducing to the herd through his daughters was of great value.

Bull No. 2. Lily's Plato. This pedigreed Guernsey bull was introduced from America. A comparison of the yields of his daughters with their dams is as follows:

Item		Milk (lb.)
Mature average yields of 14 daughters	=	4800
Mature average yields of 14 dams	=	5200
Difference: decrease	=	−400

Coming, as he did, from a well-known family of Guernseys and having been put to high grade European dams, his progeny's performances cannot be said to be good. There are obviously inherent factors for milk which are not being expressed owing to the effect of climatic conditions on animals of European types in the tropics. One of his best daughters with a mature yield of 7200 lb. was out of a Jersey-Zebu grade cow, the Indian blood apparently permitting fuller expression of the milking capacity. He sired three daughters out of European cows which were failures and were not milked.

Bull 3. King Pontiac. This pedigreed Holstein-Friesian bull was imported from a reputable strain of American Holsteins. His daughters produced the following records:

Item		Milk (lb.)
Mature average yields of 14 daughters	=	6250
Mature average yields of 14 dams	=	4300
Difference: increase	=	+1950

This creditable increase would be even more marked were it not for the low yields of three of his daughters out of European (Red Poll) dams. Omitting these, the average production of eleven daughters was 7300 lb. out of dams averaging 3600 lb. He has the honour of siring the best producer in the records of the herd, Norbrook II (Pl. I, fig. 5) with a mature yield of 16,700 lb. out of a dam which was of one-half Zebu blood. Of six "failures," four were pure European cross-breds.

Bull No. 4. Sir Pauline Homestead. This bull was also imported from a noted strain of American Holstein-Friesians.

Item		Milk (lb.)
Mature average yields of 9 daughters	=	7050
Mature average yields of 9 dams	=	7325
Difference: decrease	=	−275

It is seen that he almost managed to maintain the relatively high average of 7325 lb. of milk. Three "failures" were sired by him, all out of high grade European cows with no Zebu blood.

Bull No. 5. Sanhiwal Pusa. This bull was imported from India in 1920. He is of the Montgomery breed of Zebu cattle, specially selected for milk. His dam gave in her best lactation 6312 lb. of milk, although the average figure for the breed is 2500–3000 lb.

Item		Milk (lb.)
Mature average yields of 10 daughters	=	5700
Mature average yields of 10 dams	=	4730
Difference: increase	=	+970

The increase in constitution brought about by this bull was even more remarkable than the milk yields of his daughters, although the yields of some of them seem to demonstrate the correlation between the two increases. His two best daughters gave yields of 8280 and 8000 lb. of milk from European dams giving 3550 and 5150 lb. The high yields of the daughters suggest the re-expression of factors for milk present in the European parent although incapable of expression because of lack of constitution.

The other and less satisfactory side of the picture is concerned with the number of daughters which have to be classed as "failures"—seventeen in number. Whereas in the case of European failures the cause is that of a constitutional inability to produce milk, in the case of these Zebu failures it was the impossibility of securing the milk produced which was the problem. Bad temper, dangerous kicking and refusal to let down milk unless when being suckled combined to render these animals useless for dairy purposes. To control this undesirable temperament Hammond suggests the selection of a Zebu cross bull out of a tractable cow, special attention being paid to the latter animal's temper, as it is in the female that such characters are displayed.

Bull. No. 6. Pogis 99 of Hood Farm 40. This bull can lay claim to membership of the most outstanding family of American Jerseys, from the production standpoint. He was used extensively with the following results:

Item		Milk (lb.)
Mature average yield of 36 daughters	=	5970
Mature average yield of 26 dams	=	4750
Difference: increase	=	+1220

This increase on such a number of daughters can only be attributed to the noted prepotency for milk yield possessed by this strain of Jerseys. The average yield of seventeen pure Jerseys sired by him is 5740 lb. and that of

Jersey-Zebu grades is 6600 lb. While some of the former yields are quite satisfactory, it must be borne in mind that the animals in many cases showed signs of deterioration in constitution, actually limiting their productive lives or making for a next generation still less fit. Both cows, figs. 1 (1st generation in Jamaica) and 2 (2nd generation in Jamaica) on Pl. I, are daughters of this bull, and although the cow illustrated by fig. 2 has a mature record of 8000 lb. of milk it is obvious that she cannot stand up long to the strain of such a yield.

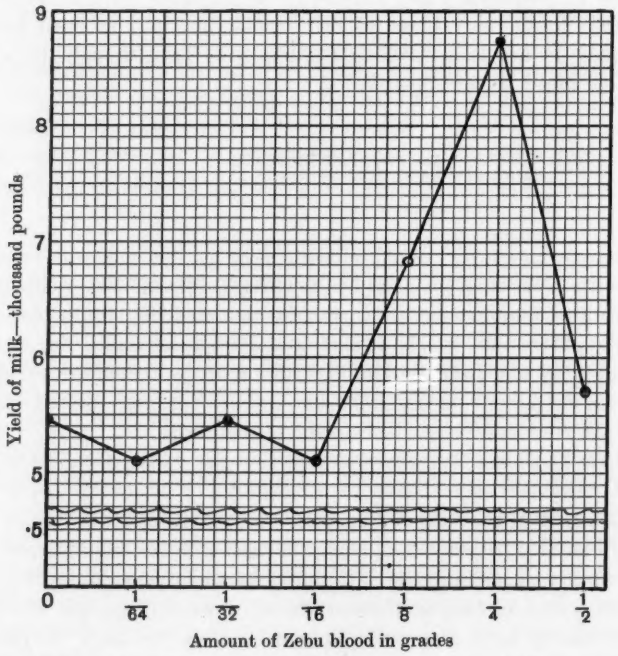

Fig. 2. Milk yields of different grades in crosses between Zebu and European cattle.

B. *Analysis of yield on percentage of Zebu blood basis.*

The milk yields of the daughters of these bulls are now grouped according to the percentages of European and Zebu blood possessed by them—a grouping justified by Lush (7). Table III gives a summary of these yields, stated as a mature average for each breed and its respective grades, and as a weighted average for the grades of all breeds. Fig. 2. is the graphical representation of this weighted average. The number of "failures" is listed in each grade column and finally stated as a percentage of the "successes" or cows milked.

From the table it is seen that in almost every case the average yields of the intermediate Zebu crosses exceed those of the cattle of pure European blood on the one hand and the one-half Zebu blood animals on the other. In the crosses of the breed with the highest potential range of yield one would expect

to find the relationship between yield and constitution most clearly defined, an expectation fulfilled in the present case in the range of the Holstein grades. The average yield of the Holsteins with no Zebu blood is seen to be 5700 lb. of milk, of the Holstein grades with one-half Zebu blood, 5325 lb., while the intermediate grades average 7250 lb. with one-eighth and 6580 lb. with one-thirty-secondth of Zebu blood. This trend is naturally also to be observed in the weighted average of grades of all breeds both in the table and graph. Of some importance also is the number of failures produced in each grade in relation to the number of successes. It is seen from Table III that this number varies again between the two high extremes encountered in the case of the pure European animal and the grades with one-half Zebu blood. In the former case 37·5 per cent. of the animals bred failed, in the latter, 40 per cent. It will be remembered that the causes of failure are entirely different and characteristic in each case. In the European grade, the lack of constitution prevented the production of

Table III. *Mature milk yields of cows in the herd of the Government Stock Farm, Hope, Jamaica, grouped according to the amount of Zebu blood in the crosses.*

Item	European pure and grade (no Zebu blood) (lb.)	Amount of Zebu blood in crosses					
		$\frac{1}{64}$ (lb.)	$\frac{1}{32}$ (lb.)	$\frac{1}{16}$ (lb.)	$\frac{1}{8}$ (lb.)	$\frac{1}{4}$ (lb.)	$\frac{1}{2}$ (lb.)
Holstein	5,700	—	6,580	5,300	7,250	13,350	5,325
Jersey	5,700	5,100	5,700	5,000	6,700	6,450	6,500
Guernsey	4,290	—	4,760	5,520	—	—	5,350
Weighted average	5,480	5,100	5,480	5,100	6,850	8,750	5,700
Number in group	40	1	13	19	4	6	10
Number of failures	15	0	0	2	1	1	4
Percentage of failures	37·5	0	0	10·5	25·0	16·7	40·0

milk. In the 50 per cent. Zebu, bad temperament made it impossible to get the milk from the animal.

In attempting to get a picture as fair and comprehensive as possible from the present data it is necessary to bear the following facts in mind. The European bulls introduced have in every case been representatives of high-milking strains justifying an expectancy of reasonably good performances. Their European-blooded progeny have failed to come even near to producing yields which might be looked for. Inadequate feeding might be cited as a reason for this, but the presence of animals of other constitutions in the same herd under the same conditions with records up to 16,700 lb. of milk points to some other cause. Photographs of the European cattle bred in the island show that reasonable yields may even be obtained from animals with an already declining constitution. If to this strain of mediocre milking cows of waning constitution, its large group of animals with health already so far impaired as to render them non-producers, be added, it becomes obvious how untenable the position of the European full blood really is. The cumulative effect of a constitution unsuited to tropical conditions is insurmountable within the strain.

At the other extreme, in considering the case of the group of grades with one-half Zebu blood, the presence again of a large group of failures makes the group quite uneconomic, even although the cause in this case is not so fatal. Possibly the careful selection of Zebu bulls for the docility character in their dam, or by making the cross in the reverse direction (*i.e.* European bull × Zebu cow) would surmount this difficulty. The better results obtained in the first crosses elsewhere (8, 9) would suggest that the difficulty might be overcome by these methods, while early handling of the young stock would also assist.

In the present case, however, the desirable combinations of the higher yields and the lower percentages of failures appear in the grades between the two extremes. This fact suggests that from them a strain best suited to the environment might be developed. Naturally some segregation to either type will occur, but given sufficient numbers of animals with about one-quarter to one-eighth Zebu blood and by selection within such a group, the attainment of a strain combining sufficient constitution with a satisfactory milk yield is a reasonable certainty. It is conceivable that under conditions tropically more severe than those of Jamaica it may be desirable to have still more Zebu and less European blood. The type of animal required may be like that of Pl. I, fig. 6, which has three-quarters Zebu blood, and one-quarter European, as compared with Fig. 5—three-quarters European and one-quarter Zebu.

Conclusions.

1. The influences of age and service period upon the milk yields of dairy cattle in Jamaica have been examined and correction factors to a mature yield have been evolved.

2. The influences of six bulls introduced to the island have been studied by means of the milk records of their progeny, first individually and secondly by an analysis of milk yields in grades on a percentage of Zebu blood basis. An analysis of "failures" on the same basis has also been made.

3. The European group (with no Zebu blood) is seen to possess a low average yield and a high percentage of constitutional failures. Its representatives have the inheritance to produce milk but lack the constitution to express their inheritance. The group of grades with one-half Zebu blood has a similar average yield and an equally high percentage of non-producers which might be in this case termed temperamental failures. The group possesses constitution but lacks the factors for a better milk inheritance and docility.

4. The occurrence of the highest average yields amongst the grades possessing one-thirty-secondth to one-quarter Zebu blood coupled with a lower percentage of failures in these grades, points to their being the most satisfactory medium for the development of a new breed suited to the environment.

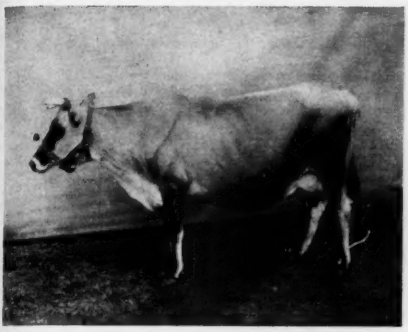

Fig. 1.

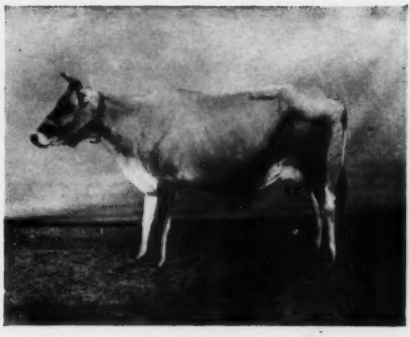

Fig. 2.

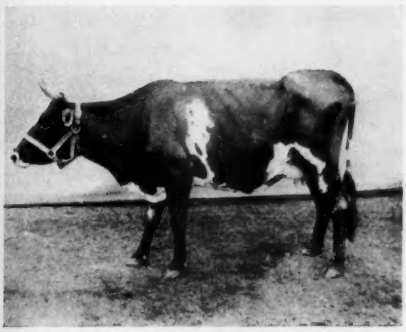

Fig. 3.

Fig. 4.

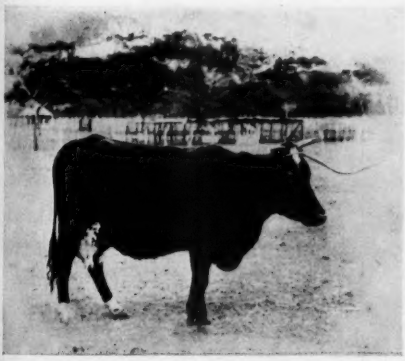

Fig. 5.

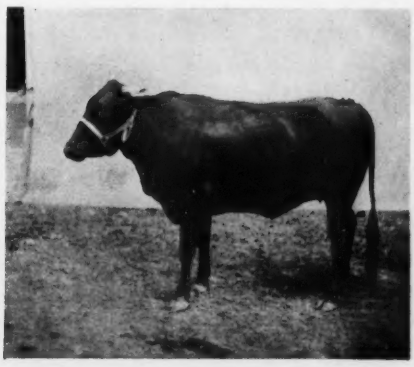

Fig. 6.

DESCRIPTION OF PLATE I

Fig. 1. 1st generation. Pure Jersey bred in Jamaica.
Fig. 2. 2nd generation. Pure Jersey bred in Jamaica showing loss of constitution.
Fig. 3. High grade Jersey cow showing effects of loss of constitution.
Fig. 4. Daughter of cow in Fig. 3 × Zebu bull. Note increase in constitution.
Fig. 5. Highest-yielding cow in herd. One-quarter Zebu. Mature record = 16,700 lb. milk.
Fig. 6. Example of animal with strong constitution (three-quarters Zebu), lacking milk-inheritance.

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42. THE YIELD AND COMPOSITION OF MILK WITH SPECIAL REFERENCE TO THE INFLUENCE OF CLIMATIC CONDITIONS AND OTHER FACTORS

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This article records the preliminary results of an investigation of some of the causes of the variations in the yield and composition of milk. The investigation involved the collection of information relating to climatic conditions and to the feeding and management of a herd of cross-bred Shorthorn cows; the analysis of the milk yielded by each cow at each milking for a period of 1 year; and the subsequent examination of the data by statistical methods.

While the main object of the investigation was to provide information as to the suitability of the present legal standards for fat and solids-not-fat, such factors as the influence of climatic conditions, the influence of the individual cow and the influence of the milker on yield and composition were also studied. The particular factors dealt with here are the influence of the season of the year and the influence of climatic conditions on the yield and composition of the milk of a herd.

Details of investigation.

This investigation was carried out on the herd of cross-bred Shorthorn cows at the Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down, between February, 1929, and February, 1930. After this date, seven cows which had almost completed lactations were continued under test until complete records were available. The Institute is licensed for the production of Grade A (T.T.) milk, and the herd is housed in a modern byre in which running water is laid on.

During the winter periods when the weather was suitable the cows were allowed out for exercise about 2 hours daily. The change over from winter to summer conditions was carried out gradually, the time the cows were out being extended until 13. v. 29, when the cows were out all day. Between 17. v. 29 and 2. x. 29 the cows were out during the night as well as during the day. Thereafter they were kept in at night and the time they were out during the day was gradually shortened until 23. xi. 29, when 2 hours' exercise daily again became the rule.

During the investigation there were no serious illnesses in the herd. On three mornings in each month the herd was kept in after morning milking for weighing, and on a few occasions it was brought in early in the afternoon for inspection by visiting parties. Milking commenced at 6.30 a.m. and 4.30 p.m. Nine milkers, some of whom were employed for part of the time only, milked the cows during the investigation. Apart from holidays or illness, each milker milked the same group of cows for 1 week and then changed in rotation to another group. Some of the part-time milkers milked only in the morning, and some only in the evening.

During the first winter period the cows were fed hay, oat straw, roots and a meal mixture. The higher-yielding cows received no straw and the lower-yielding cows no hay. The meal mixture consisted of:

	Parts by weight
Soya bean meal	1
Earth nut cake	1
Maize gluten feed	2
Maize meal	2
Crushed oats	2
Bran	1

Half the herd was given a mineral supplement[1] consisting of:

	Parts by weight
Steamed bone flour	10·0
Salt	1·5
Potassium chloride	2·0
Iron oxide	0·1
Sulphur	0·25
Potassium iodide	0·01

This mixture was fed at the rate of 2 per cent. of the weight of concentrates.

During the summer period the meal mixture remained unchanged, and where necessary, the cows were given concentrates as a supplement to grass. Thus, in June, grass alone was considered sufficient for cows yielding from 3 to 4 gallons, and higher-yielding cows received extra concentrates. In September, on the other hand, all cows giving more than 2 gallons received extra concentrates. Cows kept in during this period received fresh grass, or later, fresh vetches and oats.

When cows were kept in at night (3. x. 29 onwards), 2 lb. extra concentrates were fed. During this period green clover and cabbages were fed after milking to all cows. When clover was finished, hay and straw were resumed. When cabbage was finished, 30 lb. of turnips per head were given.

When winter quarters were resumed the meal mixture was slightly modified and in addition some of the higher yielding cows received 1 lb. of linseed cake meal.

[1] This mineral supplement has now been modified and, in addition to the constituents mentioned, contains calcium carbonate.

Data were obtained for sixty-five complete and incomplete lactations of fifty-one cows, distributed as follows:

eighteen cows were in milk when the investigation was started;

twenty-four complete lactations were recorded during the period;

twenty-three lactations were incomplete when the investigation ended.

Two cows were not put in calf, and continued in milk throughout the period. The number of cows in milk during the investigation varied from twenty-seven to thirty-five, with an average of thirty-three. Forty cows calved during the investigation.

At each milking the milk from each cow was thoroughly mixed and a 4 oz. sample taken. The unpreserved samples were despatched daily to the Chemical Research Division, Queen's University, Belfast, where tests for butterfat and specific gravity were made. Butterfat tests were made by the Gerber method, using butyrometers and pipettes which had been compared with N.P.L. apparatus. Lactometers, before use, were checked by the gravimetric method. The percentage of total solids and the percentage of solids-not-fat were calculated from the formula $T = 0{\cdot}25G + 1{\cdot}2F + 0{\cdot}14$. The total number of samples tested was just under 26,000.

Details were kept in the form of a diary of all information likely to be of use. Thermographs were installed inside and outside the byre, and were checked periodically with N.P.L. thermometers. Data relating to the temperature and humidity at the Aldergrove Meteorological Station were obtained and used to calculate the dewpoint.

The data collected included the milk yield (to the nearest $\frac{1}{4}$ lb.), the percentage of butterfat (to the nearest 0·05 per cent.), and the percentage of solids-not-fat (to the nearest 0·01 per cent.), yielded by each cow at each milking. The weight of butterfat and solids-not-fat yielded by each cow at each milking were calculated, as was the average yield of the herd at each milking. Daily yields, weekly yields and 4-weekly yields of each cow and of the herd were calculated.

Temperature, humidity and dewpoint.

Some explanation of the method of dealing with the temperature data is necessary. The maximum temperature during the day occurred usually between 2 and 5 p.m., or just before the evening milking, and the minimum temperature during the night between 4 and 6 a.m., or just before the morning milking. The milk obtained at the morning milking is secreted, therefore, during a continuous fall in temperature, and it seems reasonable to use the average temperature of the period immediately preceding the milking as the temperature at which the milk was formed. In this way, allowance is made for the possibility of high or low temperatures affecting secretion, and since the day and night temperatures are averaged separately, the difference between the averages is an indication of the range of temperature during the 24 hours. The alternative method of handling the data, that of measuring the

temperature by the range between the maximum day temperature and the minimum night temperature, is open to criticism, since no account is taken of the times at which these temperatures occur, or of the duration of the high or low temperatures.

Since milking commenced at 6.30 a.m. and 4.30 p.m., the interval between these times was taken as the day interval and the interval between 4.30 p.m. and 6.30 a.m., as the night interval. Day temperatures were obtained by averaging the thermograph readings at 8 a.m., noon, and 4 p.m., and night temperatures by averaging the readings at 8 p.m., midnight, and 4 a.m. It was found by trial that the averages of readings at these times were sufficiently close to the averages of hourly readings to serve the purpose. The means of the day and night temperatures were taken as the daily (24-hour) temperatures.

Table I. *Average monthly temperatures*, ° F.

	1929										1930	
	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Temperature outside byre:													
Day	36·5	46·0	45·9	52·4	55·4	59·6	56·8	56·6	46·7	41·4	38·3	37·1	33·8
Night	35·8	39·3	39·9	46·6	49·8	54·7	53·2	52·6	44·2	40·5	38·3	36·2	31·7
Daily	36·1	42·6	42·9	49·5	52·6	57·1	55·0	54·6	45·4	41·0	38·3	36·7	32·8
Temperature inside byre:													
Day	—	—	52·9	59·3	61·3	65·4	61·7	62·4	55·5	51·3	50·4	49·5	49·1
Night	—	—	50·6	56·0	55·7	60·3	58·0	57·7	55·0	51·8	50·3	49·5	49·4
Daily	—	—	51·7	57·7	58·5	62·8	59·9	60·0	55·2	51·6	50·3	49·5	49·2

Table I shows the average monthly temperatures inside and outside the byre. The thermograph inside the byre was not installed until 4. iv. 29.

As would be expected, there are considerable differences between the outside and inside temperatures. These differences are much greater in winter than in summer, owing to the fact that in winter the cows are in the byre for the greater part of the day and night.

In this investigation the effect of temperature is studied by taking the outside temperature only. Although there are differences between the temperatures inside the byre and those outside the byre, the close relationship of the two series may be seen from the correlations in Table II. These are high,

Table II.

Correlation* between:	$r \pm$S.E.
Daily temperature outside byre and corresponding temperature inside byre	0·9481 ±0·0038
Day " " " "	0·9404 ±0·0044
Night " " " "	0·9262 ±0·0054

* In this table the correlation coefficients are based on 321 pairs of observations. In the succeeding tables all the correlation coefficients are based on daily, day and night averages, *i.e.* on 365 pairs of observations.

considering the variations which might take place in the inside temperature. The correlation between the night temperatures is not quite so close as that between the day temperatures. This is due to the fact that the cows were indoors more often during the night than during the day. The temperature

inside the byre would be influenced by the number of cows in the byre, the extent of the ventilation, particularly at night and in cold weather, and to incidental factors, *e.g.* leaving the byre door open, visiting parties, etc.

The Hillsborough Meteorological Station was not established at the time of the investigation, and since it was thought desirable to study the variation in the yield and composition of milk in conjunction with humidity and dewpoint, hourly data supplied by the Royal Air Force Meteorological Station, Aldergrove, were used to calculate the Aldergrove average day, night, and daily temperatures and humidities, from which average dewpoints were calculated.

On comparing the Aldergrove temperatures with the Hillsborough temperatures it was found that at the former place the average temperatures were slightly higher. Compared with temperatures, the humidities were fairly constant throughout the year, and therefore the dewpoints followed very closely the trends of the temperatures. The differences in the average temperatures are no doubt due to the fact that Aldergrove is 15 miles from Hillsborough, and is situated near the shores of Lough Neagh, while Hillsborough occupies a rather exposed inland position, parts of the farm being over 500 ft. above sea-level.

Table III.

Correlation between:	$r \pm$S.E.
Outside daily temp. at Hillsborough and corresponding temp. at Aldergrove	0·9789 ±0·0015
,, day ,, ,, ,, ,,	0·9760 ±0·0017
,, night ,, ,, ,, ,,	0·9753 ±0·0017
9 a.m. humidity at Hillsborough and corresponding humidity at Aldergrove (June–November, 1930)	0·8139 ±0·0173

As Aldergrove is situated at some distance from Hillsborough, it is necessary to show, before using the Aldergrove humidity data, that the climatic conditions at the two places are similar. The correlations between the Aldergrove and Hillsborough temperatures, and between the Aldergrove and Hillsborough humidities (Table III), show that the temperatures and humidities at the two places are very closely connected. Humidity data were not collected at Hillsborough until June, 1930, and although the comparison between the humidities at the two places is made with data collected some time after the investigation had ended, it is reasonable to assume that the connection was the same during the investigation.

Owing to the voluminous nature of the actual data, it was not possible to include them here, but a study has been made of the statistical relationships of the variables by reducing all the observations to a common scale. This was done by plotting the values of $\frac{x-\bar{x}}{\sigma}$ (*i.e.* the deviation from the mean divided by the standard deviation of the series) for each variate against time.

The diagrams showed, in a very definite manner, that the milk yield was much less variable than the other variables plotted. There were, however, considerable fluctuations from day to day and from night to night in each case,

and it was apparent that the day to day variations were not always in the same direction as the night to night variations. The diagrams also showed the seasonal trends of each variable, and that the maxima and minima were reached at different times of the year. The maximum and minimum values of each variable are tabulated in Table IV from which it will be seen that the temperature was lowest in February and highest in July. The rise and fall was fairly uniform but the day to day variations were somewhat erratic. The milk yield increased until May and then decreased until August, after which the fall was very gradual to a minimum in January. If the first day of the investigation be excluded, the percentage of total solids was lowest in July and highest in December.

Table IV. *Maximum and minimum value of variables.*

	Minimum value	Date	Maximum value	Date	Range
Day temperature, ° F.	29·7	27. ii. 29	70·0	15. vii. 29	40·3
Night temperature, ° F.	25·7	10. ii. 30	64·0	15. vii. 29	38·3
Milk yield, lb. a.m.	12·45	21. i. 30	19·58	22. v. 29	7·13
,, p.m.	9·06	22. i. 30	15·87	21. v. 29	6·81
Butterfat, % a.m.	2·86	19. vi. 29	3·87	23. ix. 29	1·01
,, p.m.	3·60	12. vii. 29	4·95	2. x. 29	1·35
Solids-not-fat, % a.m.	8·60	4. viii. 29	9·28	13. iii. 29	0·68
,, p.m.	8·43	8. ix. 29	9·29	18. ii. 29	0·86
Total solids, % a.m.	11·57	11. vii. 29	12·82	19. ii. 29	1·25
,, p.m.	12·29	12. vii. 29	14·01	19. ii. 29	1·72

The variation in the percentage of butterfat over the year was fairly large. The lowest average percentage of butterfat in the morning milk was 2·86 on 19. vi. 29, and the highest 3·87 on 23. ix. 29. On sixteen occasions the morning average was below the legal standard. The evening percentage of butterfat varied from 3·60 on 12. vii. 29 to 4·95 on 2. x. 29. The percentage of solids-not-fat fell gradually and remained low during July, August and the beginning of September. The highest percentages occurred in February and March. The evening percentage of solids-not-fat was below the legal standard on five occasions.

THE CONNECTION BETWEEN MILK YIELD, BUTTERFAT AND SOLIDS-NOT-FAT.

Before proceeding to measure the influence of the season of the year and the influence of climatic conditions on yield and composition, the connection between the milk yield, butterfat and solids-not-fat yield of the herd was studied. The practical value of the results of such a study is limited, owing to the fact that the particular herd under consideration may not be representative of an "average" herd. A knowledge of the connection between these variables is, however, desirable when the influence of any external factor on the yield and composition of milk is being measured.

Table V shows that, as would be expected, there is a very close connection between the average milk yield and the average yield of butterfat, solids-not-fat and total solids. The correlation coefficients may be interpreted to mean

that the secretions of the constituents of milk are interdependent. It will be seen that the correlation between the morning yield of milk and the corresponding yield of butterfat is not so close as that between the evening yield of milk and the corresponding yield of butterfat.

The correlation between the morning butterfat yield and the corresponding solids-not-fat yield is significantly less than that between the evening butterfat yield and the corresponding solids-not-fat yield (difference $0{\cdot}1158 \pm 0{\cdot}0137$). Since the correlations between milk yield and solids-not-fat are closer than those between butterfat yields and solids-not-fat yields, it may be assumed that the solids-not-fat percentages are less subject to variation than the butterfat percentages. In this connection Gowen(1), states that the correlation between the lactation milk yield and butterfat yield for a constant solids-not-fat is practically zero and draws the inference that all correlation between milk yield and butterfat yield is due to the correlation of these variables with

Table V. *Relationships between day to day averages of herd.*

Correlation between:	$r \pm$S.E.
Milk yield a.m. and corresponding yield butterfat a.m.	$0{\cdot}8074 \pm 0{\cdot}0123$
„ p.m. „ „ p.m.	$0{\cdot}9236 \pm 0{\cdot}0052$
„ daily „ „ daily	$0{\cdot}9242 \pm 0{\cdot}0051$
Mild yield a.m. and corresponding yield solids-not-fat a.m.	$0{\cdot}9871 \pm 0{\cdot}0009$
„ p.m. „ „ p.m.	$0{\cdot}9858 \pm 0{\cdot}0010$
„ daily „ „ daily	$0{\cdot}9858 \pm 0{\cdot}0010$
Milk yield a.m. and corresponding yield total-solids a.m.	$0{\cdot}9791 \pm 0{\cdot}0015$
„ p.m. „ „ p.m.	$0{\cdot}9840 \pm 0{\cdot}0012$
„ daily „ „ daily	$0{\cdot}9869 \pm 0{\cdot}0009$
Butterfat yield a.m. and corresponding yield solids-not-fat a.m.	$0{\cdot}8030 \pm 0{\cdot}0125$
„ p.m. „ „ „ p.m.	$0{\cdot}9188 \pm 0{\cdot}0055$
„ daily „ „ „ daily	$0{\cdot}8749 \pm 0{\cdot}0083$

NOTE. The milk yields, etc., correlated in this and the succeeding tables were found by dividing the total milk yielded at a milking by the number of cows in milk at that milking.

solids-not-fat. Partial correlations, using the data in Table V, support the view that this statement may also apply in the case of the average yield of a herd. When the respective correlations with solids-not-fat yield are eliminated, the correlation between the milk yield and the corresponding butterfat yield is reduced to 0·1152 for the morning milking and 0·2684 for the evening milking, but when the effect of butterfat yield is made constant in the same way, the correlation between morning milk and solids-not-fat still remains high at 0·9625.

The correlations between milk yields and the corresponding percentages of butterfat are negative and moderately high, showing that high milk yields are associated with low percentages of butterfat (Table VI). This is a well-known phenomenon—low tests occur in the season when milk yields are high. That milk yields are high in early summer is due partly to the fact that a greater proportion of cows calve in late spring than at other seasons of the year, and high milk yields, with corresponding low percentages of fat, are due to the influence of the stage of lactation more than to any other factor. It is shown later that when the milk yield is made constant, the effect of season on butter-

fat percentage is comparatively small. The correlation between the evening percentage of butterfat and the corresponding milk yield is not so high as that between the morning percentage of butterfat and the corresponding milk yield. This is due to the closer association of the evening butterfat yield with milk yield.

Table VI. *Relationships between day to day averages of herd.*

Correlation between:	$r \pm$S.E.
Milk yield a.m. and corresponding % butterfat a.m.	−0·7430 ±0·0160
" p.m. " " p.m.	−0·4838 ±0·0270
" daily " " daily	−0·7199 ±0·0170
Milk yield a.m. and corresponding % solids-not-fat a.m.	0·0886 ±0·0352
" p.m. " " p.m.	−0·0150 ±0·0353
" daily " " daily	0·0466 ±0·0352
% solids-not-fat a.m. and corresponding % butterfat a.m.	0·0167 ±0·0350
" p.m. " " " p.m.	−0·0400 ±0·0350
" daily " " " daily	−0·0146 ±0·0350
Milk yield a.m. and corresponding % total solids a.m.	−0·5533 ±0·0246
" p.m. " " p.m.	−0·3898 ±0·0298
" daily " " daily	−0·5116 ±0·0260

The correlations between milk yields and the corresponding percentages of solids-not-fat are not significant. This is to be expected as the correlations between milk yields and the corresponding yields of solids-not-fat are almost perfect. Further, the diagrams previously referred to showed that the variation in the percentage of solids-not-fat which takes place from month to month is independent of the rise and fall of the milk yield. On the other hand, the variation from month to month in percentage of butterfat is fairly high and when variation does take place, this variation is inversely as the milk yield, and so a negative correlation between milk yield and butterfat percentage is to be expected. The negative correlation between milk yield and percentage of total solids is due to the correlation between milk yield and percentage of butterfat.

The correlations in Table VII are positive, high, and significant. They show that the connection between the tests at the morning and evening milkings and

Table VII. *Relationships between day to day averages of herd.*

Correlation between: (Previous) and	a.m. % butterfat	p.m. % butterfat	Daily % butterfat
a.m. % butterfat	+0·7270 ±0·0180	+0·5190 ±0·0260	+0·6659 ±0·0200
p.m. "	+0·5320 ±0·0250	+0·4870 ±0·0270	+0·5834 ±0·0235
daily "	+0·7110 ±0·0180	+0·4912 ±0·0270	+0·6794 ±0·0190

Note on reading table, *e.g.* +0·7270 is correlation between a.m. % butterfat and previous a.m. % butterfat.

the tests at the previous milkings is close, that is, if the test at the morning or evening milking is high, the tests at the next two milkings are also likely to be high. This result is in accord with known facts—high tests and low tests occur at certain seasons of the year, and although the tests vary from milking to milking they do not usually vary outside what might be called their "seasonal values." The connection between the morning tests is closer than that between

the morning and succeeding evening tests or that between the evening and succeeding evening tests. From this it may be concluded that the evening tests do not vary in the same way as the morning tests. The correlations between the daily tests lie mostly between the values of the morning and evening correlations.

The influence of the season of the year.

In this section the influence of the season of the year is measured by correlating temperature with the variables under investigation. Temperature is thus used as a measure of season, which is in reality a complex factor, the sum of many influences to which it would not be possible to assign a numerical value. In so far as temperature varies from winter to summer, the correlation of temperature with yield and composition is a measure of the influence of season, but where correlation exists, it does not necessarily mean that the

Table VIII.

Correlation between:				$r \pm$S.E.
Day temperature and	a.m.	milk yield on following day		0·4467 ±0·0283
,,	p.m.	,,	same day	0·4938 ±0·0268
,,	a.m.	yield of butterfat on following day		0·2578 ±0·0331
,,	p.m.	,,	,, same day	0·5034 ±0·0264
,,	a.m.	,,	solids-not-fat on following day	0·3302 ±0·0316
,,	p.m.	,,	,, same day	0·3371 ±0·0304
,,	a.m.	,,	total solids on following day	0·3275 ±0·0316
,,	p.m.	,,	,, same day	0·4356 ±0·0287
Night temperature and	a.m.	milk yield on following day		0·2645 ±0·0330
,,	a.m.	yield of butterfat on following day		0·1422 ±0·0347
,,	a.m.	,,	solids-not-fat on following day	0·1733 ±0·0343
,,	a.m.	,,	total solids on following day	0·1732 ±0·0345
Daily temperature and	daily	milk yield on following day		0·3588 ±0·0307
,,	,,	yield of butterfat on following day		0·3713 ±0·0306
,,	,,		solids-not-fat on following day	0·2877 ±0·0324
,,	,,		total solids on following day	0·3143 ±0·0319

association is due to the influence of temperature, but to the sum influence of those factors which vary with temperature.

The correlations between average temperatures and average yields of milk, fat, solids-not-fat and total solids are shown in Table VIII. Those between temperatures and milk yields are positive and moderately high. The association between high temperatures and high milk yields does not mean that high temperatures are the cause of high milk yields. The association may be accidental, and high milk yields in the season when temperatures are high may be due to the influence of any one of the factors which vary with the season of the year (*e.g.* the stage of lactation, or the nutritive value of grass in early summer).

It will be noticed that day temperature is more closely associated than the night temperature with the morning milk yield. This effect is apparent throughout this table, day temperature in every case being more closely associated with yield and composition than night temperature. A possible explanation of this is that the outside night temperature for a large part of the year is not

representative of the temperature under which the cows are kept for the period preceding the morning milking, owing to the fact that the cows are indoors at night for the greater part of the year.

The correlations between temperatures and butterfat yields are mostly smaller than those between temperatures and milk yields. The correlation between day temperature and morning yield of butterfat is only half of that between day temperature and evening yield of butterfat, therefore, in relation to temperature, morning butterfat yield varies more than evening butterfat yield. This result is due partly to the fact that the evening butterfat yield is more closely associated with the corresponding milk yield than is the morning butterfat yield. The correlations between temperatures and solids-not-fat yields are lower than those between temperatures and milk yields. The correlations between temperatures and yields of total solids are similar to those between temperature and butterfat and solids-not-fat yields.

The partial correlation between day temperature and morning yield of butterfat for a constant milk yield is − 0·1950, and that for night temperature and morning yield of butterfat − 0·1255. On the other hand, the partial correlations between day temperature and evening yield of butterfat and daily temperature and daily yield of butterfat for constant milk yields are positive, being 0·1418 and 0·1113 respectively. These four correlations, although small, point to the fact that the butterfat yield at the morning milking is depressed slightly during the season when temperatures are high in spite of the compensatory effect of increase in milk yield. Since the evening yield of butterfat is not depressed to such an extent when temperatures are high it would seem that the correlation between evening milk yield and evening yield of butterfat more than outweighs the correlation between temperature and butterfat yield, *i.e.* seasonal effects are more pronounced on the morning yield of butterfat than on the evening yield of butterfat. The fact that the correlation between morning yield of milk and butterfat is not so close as that between the evening yield of milk and butterfat supports this view.

For a constant milk yield, the correlation between day temperature and morning yield of solids-not-fat is − 0·7736 and between day temperature and evening yield of solids-not-fat − 0·7509. The size of these correlations indicates that the season of the year, as measured by temperature, has a pronounced effect on the yield of solids-not-fat or, in other words, low yields of solids-not-fat may be expected often in the season when temperatures are high. The differences between the sizes of these correlations and the corresponding ones for butterfat show that butterfat and solids-not-fat do not vary together as the seasons change. This was confirmed by studying the original data on which the correlations were based. It was seen that the percentage of butterfat fell only slightly when the temperature began to rise and in July, when the temperature was highest, the percentage of butterfat had begun to rise again. Most of the low solids-not-fat percentages, on the other hand, occurred in late summer.

The correlations in Table IX confirm the observations made on the corre-

lations in the preceding table. High temperatures are associated with low percentages of butterfat in the morning milking but the evening percentage of butterfat is not so associated, owing to the closer association between evening milk yield and evening butterfat yield. That daily percentages of butterfat are lower when temperatures are high is due to the association of high temperatures with low morning percentages of butterfat. The positive association between humidity and butterfat percentage is to be expected since, generally speaking, humidity is high when butterfat tests are also high. When milk yield is made constant the correlation between day humidity and morning percentage of butterfat becomes insignificant (− 0·0254). The correlations between dewpoints and butterfat percentages are not so close as those between temperatures and butterfat percentages. Butterfat percentages are, therefore, more closely associated with actual temperature than with dewpoint. This presumably is accounted for by the fact that in calculating dewpoints, humidity is taken into consideration. The correlations between humidities and butterfat percentages bear a different sign from those between temperatures and butterfat percentages.

Table IX.

Correlation between: and	a.m. % butterfat	p.m. % butterfat	daily % butterfat
Previous day temperature	−0·4049 ±0·0300	−0·0860 ±0·0350	−0·2899 ±0·0320
„ night „	−0·2830 ±0·0330	0·0068 ±0·0350	−0·1934 ±0·0340
„ daily „	−0·3574 ±0·0310	−0·0598 ±0·0350	−0·2374 ±0·0330
Previous day dewpoint	−0·3587 ±0·0310	−0·0244 ±0·0350	−0·1349 ±0·0350
„ night „	−0·2058 ±0·0340	0·0413 ±0·0350	−0·1102 ±0·0350
„ daily „	−0·2393 ±0·0334	0·0400 ±0·0353	−0·1275 ±0·0350
Previous day humidity	0·3325 ±0·0310	0·1791 ±0·0340	0·2936 ±0·0330
„ night „	0·1971 ±0·0340	0·1887 ±0·0340	0·2457 ±0·0330
„ daily „	0·3141 ±0·0320	0·2197 ±0·0340	0·2910 ±0·0320
Fall in temperature from previous day to next night	0·3403 ±0·0310	0·1849 ±0·0340	0·3190 ±0·0320
Rise in temperature from previous night to next day		−0·1592 ±0·0340	

Note on reading table, *e.g.* −0·4049 is correlation between a.m. % butterfat and previous day temperature.

Falls in temperature from day to night are positively associated with butterfat percentages. This merely means that large falls from day to night temperature occur when butterfat tests are high, that is, in August, September and October.

The most pronounced seasonal effect noted in the foregoing sections is the depression in summer of the yield of solids-not-fat, and therefore of the percentage of solids-not-fat, since it has been shown that the percentage of solids-not-fat over the year is independent of the milk yield. This has an important practical bearing, indicating as it does, that the low percentages of solids-not-fat which are known to occur in late summer are unavoidable. The partial correlations also show that the solids-not-fat percentage is influenced to a much greater extent by season than is the butterfat percentage. It would appear

that the main factor influencing the butterfat percentage is the size of the milk yield, and that the influence of season on the butterfat percentage is comparatively small. What seasonal influence there is on the percentage of butterfat is seen in the morning percentage but not in the evening percentage. It may be concluded, therefore, that the morning butterfat percentage varies more than the evening percentage. The difference between the lengths of the intervals between the milkings, with the consequent effect on the secretion of butterfat, is the apparent cause of this difference.

The day temperature was found to be more closely associated with yield and composition than the night temperature, and it is reasonable to suppose that day temperature is a better measure of season than night temperature, since the former more nearly approximates the actual temperature under which the cows are kept. Temperature was also found to be more closely associated with butterfat percentage than was dewpoint, and when the variation in milk yield was eliminated the humidity correlations became insignificant. Temperature, therefore, is a better measure of season than either dewpoint or humidity.

The results obtained in this investigation as regards the seasonal variation in butterfat percentage agree fairly well with the results obtained by Tocher (2), who found that butterfat percentages were highest in September. Bartlett (3) found that variability of yields of milk and fat tended to be higher during the summer than during the winter months. In this investigation the butterfat percentage was found to be more variable during periods of high temperature, *i.e.* in summer.

In concluding this section it should be pointed out that certain other factors, which are known to have an influence on yield and composition, have not been taken into consideration, in some cases owing to the difficulty of assigning a numerical value to them, or, as in the case of the intervals between the milkings, because the factor remained constant throughout the investigation. It was thought advisable to deal in the first instance with the average yield and composition of the milk of the herd rather than with the milk of individual cows. In this way the influence of the individuality of the cow as a factor influencing the variables is largely eliminated, and the general influence of a factor is seen rather than its influence in a particular case. Finally, it is realised that the seasonal conditions in Northern Ireland are not strictly comparable with those of say, southern England, and hence the results may not in every case agree with those of other workers.

The influence of variations in temperature on yield and composition.

In this section the influence of changes in temperature on yield and composition is measured by correlating the changes in temperature from day to day and from night to night with the subsequent changes in yield and composition from milking to milking. Thus temperature is here considered as a

factor influencing the variables, and the main object of this part of the work was to find how the percentage of butterfat reacted to large changes in temperature during the period preceding the milking.

It is possible that the butterfat percentage may fluctuate more in one season than another. If this were the case, the correlation between temperature and the changes in the subsequent percentages of butterfat, irrespective of the direction of the changes in the latter, should be significant. This correlation was determined for day temperature and morning percentage of butterfat and found to be $0{\cdot}1801 \pm 0{\cdot}0343$, indicating that variations in butterfat percentages may be greater when temperatures are high than when they are low, *i.e.* the butterfat percentage tends to fluctuate to a greater degree in summer than in winter.

The correlations between changes in temperature and changes in milk yield are not significant (Table X). This means that although both temperature and milk yield vary from day to day, they do not do so in unison; in

Table X.

Correlation between:	$r \pm$S.E.
Change in night temperature and change in a.m. milk yield on following day	$0{\cdot}0557 \pm 0{\cdot}0354$
" day " " p.m. " same day	$0{\cdot}0085 \pm 0{\cdot}0353$
" daily " " daily " following day	$-0{\cdot}0058 \pm 0{\cdot}0355$
Change in night temperature and change in a.m. butterfat yield on following day	$-0{\cdot}0779 \pm 0{\cdot}0352$
" day " " p.m. " " same day	$0{\cdot}0052 \pm 0{\cdot}0354$
" daily " " daily " " following day	$-0{\cdot}1762 \pm 0{\cdot}0343$
Change in night temperature and change in a.m. solids-not-fat yield on following day	$-0{\cdot}0130 \pm 0{\cdot}0355$
" day " " p.m. " " same day	$-0{\cdot}0182 \pm 0{\cdot}0355$
" daily " " daily " " following day	$-0{\cdot}0570 \pm 0{\cdot}0353$
Change in night temperature and change in a.m. total-solids yield on following day	$-0{\cdot}0399 \pm 0{\cdot}0355$
" day " " p.m. " " same day	$-0{\cdot}0966 \pm 0{\cdot}0351$
" daily " " daily " " following day	$-0{\cdot}1184 \pm 0{\cdot}0350$

other words, a rise in temperature is not accompanied by either a rise or a fall in milk yield, or, put in another way, the diurnal variations of the milk yield of a herd are independent of temperature changes. Since butterfat yields are positively associated with milk yields, no correlation between changes in temperature and changes in butterfat yield need be looked for.

For the same reason no correlation is to be expected between changes in temperature and changes in the yield of solids-not-fat at the succeeding milkings, or between changes in temperature and changes in the yield of total solids at the succeeding milkings.

Turning to Table XI, it is seen that changes in temperature are negatively associated to a very slight extent with changes in the percentage of butterfat at the morning milking but not with the changes at the evening milking. There is a tendency, therefore, for a rise in temperature to depress the morning percentage of butterfat and for a fall in temperature to raise it. That this does not hold in the case of the evening butterfat test is further evidence that the morning and evening percentages of butterfat do not vary in the same manner. The correlation between changes in daily temperature and the changes in the

succeeding daily percentages of fat is higher than the correlations between changes in day and night temperatures and changes in the morning percentages of butterfat, but the difference is too small to warrant the suggestion that a depression of the morning percentage of butterfat is accompanied by a depression in the subsequent evening percentage of butterfat.

The correlations between the changes in dewpoint and the subsequent changes in percentages of butterfat are lower than the corresponding ones for changes in temperature and changes in percentage of butterfat. Therefore, changes in actual temperature are more associated with changes in percentage of butterfat than are changes in dewpoint. It follows that changes in humidity are less associated with changes in percentages of butterfat than are changes of temperature. This is seen to be the case.

There is no correlation between the change in temperature from day to night and the change in percentage of fat from one milking to another (that is, large changes in temperature from day to night do not appear to be associated

Table XI.

Correlation between change in	and	a.m. % butterfat	p.m. % butterfat	daily % butterfat
Change in previous day temperature		−0·1325 ±0·0350	−0·0080 ±0·0350	−0·1970 ±0·0340
,,	night ,,	−0·1420 ±0·0340	0·0200 ±0·0350	−0·1939 ±0·0340
,,	daily ,,	−0·1480 ±0·0350	0·1818 ±0·0342	−0·2084 ±0·0340
Change in previous day dewpoint		−0·0881 ±0·0350	−0·0811 ±0·0352	−0·0448 ±0·0354
,,	night ,,	−0·1548 ±0·0350	−0·0869 ±0·0352	0·1528 ±0·0346
,,	daily ,,	−0·1437 ±0·0347	−0·0089 ±0·0355	−0·1296 ±0·0349
Change in previous day humidity		−0·0362 ±0·0350	−0·0186 ±0·0350	0·0001 ±0·0350
,,	night ,,	−0·0985 ±0·0350	0·1191 ±0·0349	−0·0108 ±0·0353
,,	daily ,,	−0·1012 ±0·0350	0·0632 ±0·0353	−0·0289 ±0·0353
Fall between previous day and night temperature		−0·0623 ±0·0350	—	−0·0731 ±0·0353

Note on reading table, *e.g.* −0·1325 is correlation between change in a.m. % butterfat and change in previous day temperature.

with changes in percentage of butterfat in any particular direction). In order to see if changes in temperature, irrespective of the direction of the changes, were associated with changes in percentage of butterfat, the correlation between the changes in day temperature, omitting the algebraic signs of the changes, and the changes in the succeeding morning percentage of butterfat was determined and found to be − 0·1235 ± 0·0349. This correlation, which is barely significant, indicates that large changes in day temperature are not to any extent associated with decreases in the morning percentage of butterfat. The corresponding correlation for night temperature is not significant.

There is a slight negative correlation between changes in temperature and the changes in the succeeding percentages of solids-not-fat (Table XII). In this case the correlation between the daily changes in temperature and percentage of solids-not-fat is not significant, which means that falls in the morning percentage of solids-not-fat are more often accompanied by rises than falls in the evening percentage of solids-not-fat. Thus there seems to be a

compensatory effect between the morning and evening percentage of solids-not-fat. As regards the correlations between changes in temperature and changes in the succeeding percentages of total solids, these may be said to be due to the corresponding correlations between changes in temperature and changes in percentage of butterfat and solids-not-fat.

Summing up this section, it may be said that the correlations between the changes, if not insignificant, are not much above the border line of significance. Changes in temperature, therefore, are not the chief cause of the diurnal variations in yield and composition. Indeed it can be said that the diurnal variations in milk yield, butterfat yield and solids-not-fat yield are independent of temperature changes, and when the fat and solids are expressed as percentages of the milk yield, the correlations between the changes in these percentages and the corresponding changes in temperature are so small as to render their significance doubtful. What little significance there is in the correlation points to a slight depression in the morning percentage of butterfat

Table XII.

Correlation between:							$r \pm$S.E.
Change in night temperature and change in a.m. % solids-not-fat on following day							$-0{\cdot}1902 \pm 0{\cdot}0346$
,,	day	,,	,,	p.m.	,,	same day	$-0{\cdot}1492 \pm 0{\cdot}0346$
,,	daily	,,	,,	daily	,,	following day	$-0{\cdot}0856 \pm 0{\cdot}0353$
Change in night temperature and change in a.m. % total solids on following day							$-0{\cdot}2145 \pm 0{\cdot}0339$
,,	day	,,	,,	p.m.	,,	same day	$-0{\cdot}0655 \pm 0{\cdot}0353$
,,	daily	,,	,,	daily	,,	following day	$-0{\cdot}2223 \pm 0{\cdot}0337$

when the temperature rises. The evening percentage of butterfat does not react in this way. The size of the correlations also indicates that large changes in temperature from day to night are not associated with changes in the butterfat percentage in any one direction, but large changes in the day temperature are to some extent associated with decreases in the morning percentage of fat. Finally, there would seem to be a slight inverse relationship between change in temperature and change in percentage of solids-not-fat. It is possible, however, that with individual cows and different methods of analysing the data, that temperature would be found to have a more pronounced influence on yield and composition.

The results, in so far as they may be regarded as significant, confirm the conclusions of Ragsdale and Brody (4), Hayes (5), and Weaver and Matthews (6), rather than those of Lloyd (7), and Campbell (8), and are not in agreement with the conclusions of Bender (9).

Summary.

This article records the preliminary results of an investigation of some of the causes of the variations in the yield and composition of milk. The influence of the season of the year, and the influence of climatic conditions on the average yield and composition of the milk of a herd, are discussed.

The relationships between the data are studied by means of simple correlation and also by means of partial correlation. The temperatures used are the

average temperatures for the periods between the milkings. Thus the day temperature is the average temperature of the interval between the morning and evening milking, and the night temperature is the average temperature of the interval between the evening milking and the next morning milking. The daily temperature is the average of these two temperatures.

The influence of the season of the year is measured by correlating temperature with yield and composition, and the correlation coefficients so found are interpreted in terms of the sum influence of all the factors which vary with season. The influence of changes in temperature, as such, on diurnal variations in yield and composition is measured by correlating the changes in temperature between the periods preceding the milkings with the changes in the average yield and composition of the milk at the subsequent milkings.

The relationships between the secretion of milk as a whole, the secretion of solids-not-fat and the secretion of butterfat, are discussed. It is shown that butterfat yield is more variable than either milk yield or solids-not-fat yield; that variations in the butterfat percentage are due mainly to variations in the milk yield; that the morning percentage of butterfat varies more than the evening percentage of butterfat; and that butterfat percentage and solids-not-fat percentage do not vary together.

It is suggested that day temperature is a better measure of the influence of the season of the year than night temperature. It was found that the season of the year exerts a marked influence on the percentage of solids-not-fat. During the summer period the percentage of solids-not-fat is depressed. On the other hand, the effect of the season of the year on the percentage of butterfat is comparatively small. The morning percentage of butterfat is more affected by seasonal influences than the evening percentage of butterfat.

The diurnal variations in milk yield and solids-not-fat yield are independent of temperature changes. Diurnal variations in temperature affect the morning percentage of butterfat but not the evening percentage of butterfat, and also the morning and evening percentages of solids-not-fat.

The authors wish to acknowledge with thanks the helpful advice and criticism received from Dr J. F. Tocher of Aberdeen, and the assistance of Mr J. M. Shimmon, of the Chemical Division, Queen's University, who plotted the correlation tables.

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43. NORMAL GROWTH OF DAIRY CATTLE

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(With 3 Charts.)

The study of growth is of interest to scientists as well as to those who keep animals for profit or pleasure. The literature on the subject is extensive, but the information published regarding the growth of dairy cattle in this country is somewhat disjointed and tends to cover only short sections of the growing period.

In order that the results of experiments may be viewed by comparison with normal animals of similar age, it is helpful to have data showing complete normal growth curves of dairy stock. This is particularly useful as a check on control animals, and if normal growth under a given set of conditions of feeding and management is known, it may, for certain experiments, be possible to dispense with control animals altogether. Standard or normal growth curves may be regarded as a foundation for experimental work, but they also have some use from a purely practical point of view: most experienced farmers can judge whether an animal is large or small for its age, and carry in their minds a standard size at which young stock should be bred for the first time. Younger men, however, do not always have the experienced eye, and written standards by which judgment may be tested are of great assistance.

Material.

During the last 10 years considerable data have been collected regarding the sizes of young dairy stock kept at the National Institute for Research in Dairying, Reading. The records include the weights and measurements of various parts of the body, throughout the growing life of the cattle. The intervals at which the records were taken have varied slightly from time to time, but as a general rule, calves were weighed weekly, other animals monthly, and measurements were taken at intervals of 3 months.

The methods of taking measurements were similar to those described in another article (1), but a brief outline is given below of the three measurements considered here:

Methods of recording height at withers needs no description. Depth of chest was measured by calipers and is the vertical distance between the top of the backbone and the under surface of the body just behind the front legs. Length of body represents the distance from a point directly above the rear extremities of the pin-bones to the forward corners of the shoulder-blades lying alongside the backbone.

The breed, feeding and management of the young cattle.

All the cattle dealt with in this article are non-pedigree Shorthorns, and represent stock purchased in 1921 at markets and farm sales and the progeny of these animals by pedigree dairy Shorthorn bulls. The majority of the calves were removed from their dams at 4 days old and fed about 40 gallons of whole milk during the first 6 weeks of their lives. Milk was then substituted by a mixture of cakes and meals fed dry, together with hay and water. Calves born during the autumn and early winter (till about mid-January) were turned on to pasture during their first summer, but those born after mid-January were not usually allowed on pasture till their second summer. Animals under 15 months old were usually wintered in covered yards, but other animals were wintered out on a dry pasture. The grassland available for young stock was not of first quality, some being low-lying wet land and some on a gravelly soil. The rations fed in winter were probably definitely better than those fed to young stock on most dairy farms in the south of England. So far as possible, the young stock were maintained in moderate condition throughout the year, and, except when special experiments were carried out, the heifers were mated at what was believed to be an average size for the breed.

Thirty-eight of the animals used for this study possessed a complete lifetime record, and the measurements of these were compared and verified with about fifty other animals of which records of size were known for sections of their lifetime.

Growth curves.

Table I and Charts 1 and 2 give a summary of the growth of dairy Shorthorn cattle up to the age of 5 years, after which time there is very little change in size, although live weight may increase slightly beyond this age. The four columns of Table I, headed "Size expressed as percentage of size at 5 years," give an indication of the relative rates of growth of the various parts of the body. It will be seen that length and depth of the body increase at approximately the same rates, the figures at 6 months being 63·1 per cent. and 63·4 per cent. respectively, and at 18 months 83·1 per cent. 82·2 per cent. Growth in height takes place at a much lower proportional rate, owing to the small increase in length of leg. The leg of the calf is relatively much longer than that of the mature cow.

All the curves on Chart I show the rate of growth to be very rapid from birth to 6 months of age; after this time there is a gradual decrease, till finally growth ceases altogether.

Chart 2, showing the changes in live weight, needs special comment. The general trend of the curve up to 21 months of age is a straight line, the average daily increase in live weight being about $1\frac{1}{8}$ lb. per day. This constitutes a great asset when the daily live-weight increase is used as a basis for the comparison of experimental results. Moreover because the daily live-weight

increase is fairly constant, direct comparison is possible even though the animals vary somewhat in size. This is not the case if a measurement such as depth of chest is used as the criterion of experimental results, because the normal growth rate in size is decidedly lower for older animals.

From birth to 6 months of age, however, the average live-weight increase per day is not constant, but tends to show a progressively greater increase per day. Most of the calves used as the data for Chart 2 received no milk after 6 or 8 weeks of age, but if milk had been continued to 6 months of age the shape of the curve would have been slightly different. This matter is discussed later under the heading "relative growth rate."

Table I. *Normal size of dairy Shorthorn cattle from birth to 5 years of age.*

Age (months)	Farrow live weight (lb.)	Height at withers		Length of body		Depth of chest		Size expressed as percentage of size at 5 years				Ratio height over depth
		(in.)	(cm.)	(in.)	(cm.)	(in.)	(cm.)	Weight	Height	Length	Depth	
Birth	90	29·5	74·9	24·8	63·0	11·8	30·0	7·5	56·1	42·0	40·4	2·50
3	193	34·7	88·1	31·3	79·5	15·4	39·1	16·1	66·0	53·1	52·7	2·25
6	330	39·3	99·8	37·2	94·5	18·5	47·0	27·5	74·7	63·1	63·4	2·12
9	425	42·1	106·9	41·8	106·2	20·1	51·1	35·4	80·0	70·8	68·8	2·09
12	510	44·6	113·3	44·6	113·3	22·0	55·9	42·5	84·8	75·6	75·3	2·03
15	620	46·2	117·3	46·8	118·9	23·1	58·7	51·7	87·8	79·3	79·1	2·00
18	720	47·6	120·9	49·0	124·5	24·0	61·0	60·0	90·5	83·1	82·2	1·98
21	800	48·7	123·7	51·0	129·5	25·0	63·5	66·7	92·6	86·4	85·6	1·95
24	860	49·7	126·2	52·4	133·1	25·7	65·3	71·7	94·5	88·8	88·0	1·93
27	905	50·5	128·3	53·8	136·7	26·3	66·8	75·4	96·0	91·2	90·1	1·92
30	965	51·1	129·8	54·8	139·2	27·0	68·6	80·4	97·1	92·9	92·5	1·89
33	1025	51·3	130·3	55·4	140·7	27·4	69·6	85·4	97·5	93·9	93·8	1·87
36	1045	51·5	130·8	56·0	142·2	27·7	70·4	87·1	97·9	94·9	94·9	1·86
39	1055	51·6	131·1	56·3	143·0	27·8	70·6	87·9	98·1	95·4	95·2	1·86
42	1075	51·7	131·3	56·8	144·3	28·0	71·1	89·6	98·3	96·3	95·9	1·85
45	1100	51·9	131·8	57·2	145·3	28·3	71·9	91·7	98·7	96·9	96·9	1·83
48	1130	52·1	132·3	57·7	146·6	28·5	72·4	94·2	99·0	97·8	97·6	1·83
51	1155	52·3	132·8	58·2	147·8	28·8	73·2	96·2	99·4	98·6	98·6	1·82
54	1170	52·4	133·1	58·6	148·8	29·0	73·7	97·5	99·6	99·3	99·3	1·81
57	1185	52·5	133·4	58·9	149·6	29·2	74·2	98·7	99·8	99·8	100·0	1·80
60	1200	52·6	133·6	59·0	149·9	29·2	74·2	100·0	100·0	100·0	100·0	1·80

A difficulty which is encountered in stating the normal live-weight increase of dairy cattle is the relationship of pregnancy to live weight. Shorthorn cattle are usually mated at about 2 years old, and after this time there is some difficulty in stating how much of the live-weight increase is due to pregnancy, and how much is normal growth of the parent. In order to allow for this, the average live weights of all "in calf" heifers were found at definite intervals before calving, and the curve prepared from these gave an excellent idea of the live-weight increase due to pregnancy. Assuming the first calving date to be at 33½ months of age, the section of the chart shown as a broken line gives the probable live weights of a pregnant animal, while the unbroken line shows a farrow animal.

In a previous article by Bartlett (2) the live weights of cows in a neighbouring herd are shown during the second and subsequent pregnancies, and a remarkably close agreement is found between the live weights of the two sets of

animals about 10 months before their second calving date. In both cases the figure is almost exactly 1060 lb.

After the first calving the influence of pregnancy on live weight has been disregarded in Chart 2, and the figures are based on the live weight of farrow cows only.

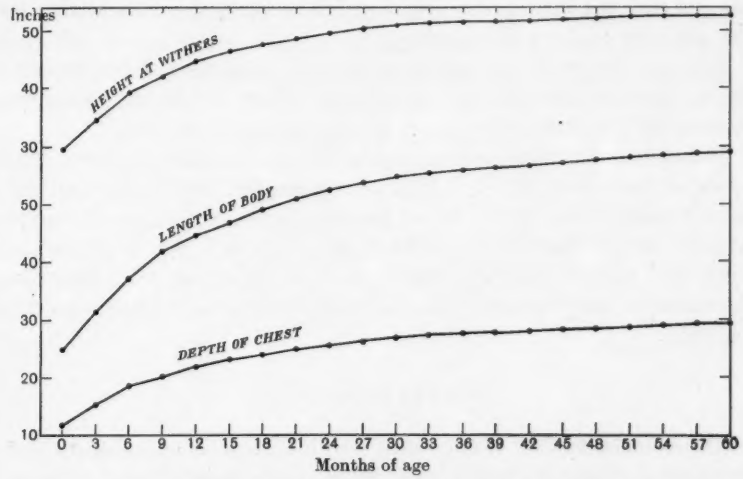

Chart 1. Showing normal growth curves of dairy Shorthorn cattle.

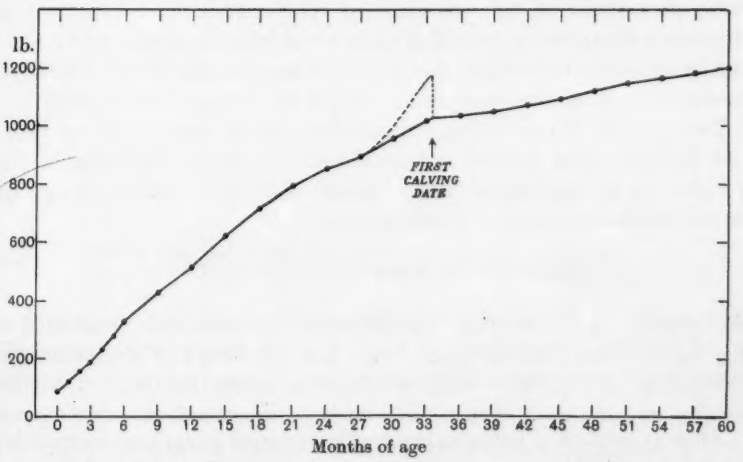

Chart 2. Showing normal growth in live weight of dairy Shorthorn cattle.

Brody and Ragsdale have concluded(3) that in the dairy cow there are two post-uterine growth cycles with maxima at about 5 months and 20 months of age. Chart 2 tends to corroborate this conclusion, particularly with regard to rapid growth at about 5 months old. It is believed, however, that the

unevenness noticeable in the curve from 18 to 24 months of age, is wholly or partially a seasonal effect, because it has been found that the curves of spring and autumn-born calves do not behave in the same manner. This point is discussed further in the last paragraph. The measurements shown on Chart 1 give little or no evidence of similar growth cycles, but it is possible that some of the finer points are obscured because of comparatively long intervals between dates of measuring.

Eckles(4) has published records of growth in height and live weight of average American Holstein and Jersey cattle. His results for the former breed compare fairly well with the records for Shorthorns shown here.

It may be noted that a certain number of records of size of growing Guernsey cattle have been collected at this Institute, but the numbers measured scarcely warrant the publication of average figures. The calves of Guernsey cattle are smaller than Shorthorns, they grow more rapidly in height for about a year and reach a smaller mature size at an earlier age than Shorthorns. The claims to early maturity as an asset of the Guernsey breed is no doubt justified.

Relative growth rate.

The common method of expressing growth in pounds or inches per day or per month is simple and usually quite satisfactory. In some cases, however, it is not the best method, particularly when comparison is needed between plants or animals of different sizes. Several writers have explained the advantages of expressing growth at a relative or percentage rate, which can be done by regarding the weight of an animal at any one time as representing the amount of "capital" and increases in weight as "interest" on capital.

The methods of calculating instantaneous growth rate tended to present some difficulty until clarified and simplified by Fisher(5). The formula given by Fisher for calculating the relative growth rate over a period during which the rate remains constant is as follows:

$$\text{Relative growth rate} = \frac{\log_e m_2 - \log_e m_1}{t_2 - t_1},$$

where m_1 and m_2 are the weights of the animal or plant at the beginning and end of the period respectively and $t_2 - t_1$ is the length of the period. The instantaneous percentage growth rate is found by multiplying the result by 100.

An easy method of determining whether relative growth is constant is to plot the live weights on graph paper in which the vertical axis is divided by a logarithmic scale and the horizontal axis by inches or centimetres.

The top left half of Chart 3 illustrates the use of this paper. On it are plotted the weekly live weights of two groups of calves. Lot 1 was a heavily fed group and received milk until nearly 6 months old. Lot 2 was fed an average diet and the milk ration ceased at about 10 weeks old. A straight line can be drawn through the live weights of lot 1 from the 4th to the 21st week, indicating a constant percentage growth rate throughout this period (5·3 per cent.). The changes in live weight of lot 2 are followed by two straight lines joining at the 10th week. This shows an abrupt change in percentage growth rate at

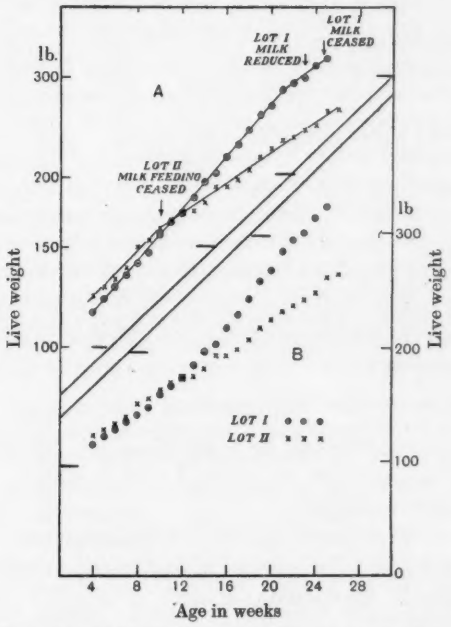

Chart 3. Showing weekly live weights of two groups of calves plotted: *A* on semilog graph paper; *B* on ordinary arithmetical graph paper.

the 10th week, the calculated figures for the two periods being, 4th to 10th week (milk diet) 4·73 per cent., and 10th to 26th week (no milk) 3·0 per cent. The abrupt change in growth rate which occurs with lot 2 on ceasing milk feeding illustrates that the changes from one growth cycle to another (noted by Brody (6)) may result from changes in diet apart from the normal tendency of animals to change in growth rate with advancing age.

On the other hand, the curve for lot 1 is a straight line up to the 21st week, when a definite break occurs, although there was no change in the diet till a month later. In this case the break would appear to be associated with a change within the animals rather than with the diet, and it also happens that this break occurs at about the age noted by Brody as the peak of the first post-

uterine growth cycle. The occurrence of these growth cycles forms the basis for the autocatalytic theory of growth first elaborated by Robertson (7) and supported by Brody and his colleagues. Hammond (8), however, disagrees with this theory and contends that growth cycles are controlled by nutrition rather than by changes in the nucleus or cytoplasm of the tissues. The evidence here supports the generally accepted view that nutrition influences growth cycles, but it also tends to corroborate the findings of Brody and Ragsdale (3) that in dairy cattle there are growth cycles which are controlled by factors other than nutrition and environment. It is hoped that further analysis of the data may throw a more definite light on this important subject.

The right half of Chart 3 shows the same figures plotted on ordinary arithmetical graph paper and the resulting curves will be seen to be somewhat confusing compared with the orderly array on the semilog paper on the left. Alternately it might be wrongly concluded from the right-hand graph that because lot 2 increased in live weight at approximately a constant rate per week, the increases were as good as could be expected. The point which it is desired to make quite clear is, that if the growth rate maintains a constant rate per day, *e.g.* 1 lb. per day, the "daily live weight increase method" may be satisfactory, but if the relative or percentage growth rate maintains a constant rate over a period, then the formula on p. 314 is a more accurate means of expressing it, the latter conditions are likely to obtain in experiments on calves under 6 months of age.

SUMMARY.

1. Normal curves of growth for dairy Shorthorn cattle from birth to 5 years of age are presented. These include height at withers, length of body, depth of chest and live weight.

2. The expression of growth by the "Relative growth rate" method is discussed and illustrated. The method is particularly suitable for expressing the growth of calves under 6 months of age.

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ABSTRACTS AND REVIEWS

BACTERIOLOGY.

General.

C. S. R. Ayyar. **The use of small additions of acid for increasing the germicidal action of E.C. on bacterial spores.** *Agric. J. India*, **25**, 213–19, 1930. (*Chem. Abst.* **25**, viii, 1865, April 20, 1931.)

Tests on a hypochlorite solution E.C. (containing 2 per cent. of available chlorine), showed that a mixed solution containing 0·2 per cent. E.C. and 0·05 per cent. *N* citric acid destroyed the spores of *B. subtilis*, *B. albolactus* and *B. mesentericus* in 30 min. With E.C. alone (0·2 per cent. solution) the spores were not destroyed after 60 min., although the numbers were considerably reduced. M. I. Christian

G. I. Wallace and F. W. Tanner. **Effect of heat on mould spores.** *Proc. Soc. Exp. Biol. and Med.* **28**, ix, 970–2, June 1931.

The authors have studied the heat resistance of spores of *Aspergillus niger*, *Oidium lactis*, *Mucor mirus*, *Rhizopus nigricans* and *Alternaria solani*. Suspensions in 1, 3 and 6 per cent. NaCl solution, 10, 25 and 50 per cent. sugar solution, juice from cherries in syrup, and distilled water were used for the tests. The results show that 60° C. for 5 min. is usually sufficient to ensure complete destruction of the spores. In some cases *Aspergillus niger* and *Oidium lactis* proved exceptional, since resistances of 60° C. for 10 min. and 30 min. respectively were recorded. Neither the sugar nor salt solution appears to exert a protective or destructive influence. M. I. Christian

H. Fink and F. Weinfurtner. **Staining of yeast cells with methylene blue and its relation to hydrogen-ion concentration and permeability.** *Woch. Brau.* **48**, 159–62, 1931. (*J. Inst. Brewing*, **37**, vi, 328, June 1931.)

Yeast cells are reported to take methylene blue more readily in alkaline than in acid solution. In the presence of sugars, normal living cells take the stain like dead cells, but show no loss of reproductive capacity. Dextrose, laevulose, cane sugar and galactose are about equally effective in increasing the permeability, whilst maltose is less so. These results indicate the need for revision of the methylene blue test for yeast. M. I. Christian

D. Müller. **Untersuchungen über Oxydasen in getöteten Essigbakterien. I.** (Oxydases in dead acetic bacteria. I.) *Biochem. Z.* **238**, 253–67, August 1931.

Acetone treated deposits of *B. pasteurianum* (*Acetobacter pasteurianus*) absorb oxygen slowly; addition of alcohols increases this uptake enormously. Ethyl alcohol is oxidised to acetic acid and *n*-propyl alcohol to propionic. Isopropyl alcohol is oxidised quantitatively to acetone, but this oxidation is inhibited by heating the preparation at 95° C. for 30 min. J. G. Davis

C. E. A. Winslow and E. T. Haywood. **The specific potency of certain cations with reference to their effect on bacterial viability.** *J. Bact.* **22**, i, 49–69, July 1931.

This paper presents a detailed study of specific cation effects on a strain of *E. coli*. Considering the potency of Na as 1, the following figures represent the activity of other cations: K 1, Li 3, Ba 5, Mg 9, Ca 12, Mn 400, Zn 700, and Cd 3000. All cations stimulate growth in low concentrations and inhibit at higher levels.

The authors suggest that the phenomena of specific potency agree with Bancroft's

theory of disinfection, according to which disinfection is due to the coagulation of the cell colloids, the decreasing stability of which in the initial phases is related to the stimulation observed. J. G. Davis

P. W. Wilson and E. D. Kullmann. **A statistical enquiry into methods for estimating numbers of Rhizobia.** *J. Bact.* **22**, i, 71–90, July 1931.

Various ways of estimating bacterial numbers were employed. Variability resulting from the use of only one plate in a series can be partly eliminated by using three or more plates, of which any one markedly abnormal may be rejected without prejudice to the results. The best results were secured by the use of the Petroff-Hausser counting chamber. Results are treated statistically. (Although *Rhizobium* is an organism requiring rather special conditions for growth, the methods used in this paper may be studied with profit by those interested in methods of estimating bacterial numbers in other species.) A. T. R. Mattick

T. R. Vernon. **An improved type of moist chamber for studying fungal growth.** *Ann. Botany*, **45**, 733–7, October 1931.

About 3 drops of a 2 per cent. agar medium are added to a sterile slide at a temperature of 80–90° C. When the medium is about to gel a sterile cover-slip is placed on it and gentle pressure used to flatten it. When the medium has set, the cover-slip is removed and the disc cut in half with a sterile knife or safety razor blade. One half is then pushed aside, one of the cut surfaces inoculated and the whole covered with a sterile cover-slip. The preparation is incubated in a moist atmosphere. The entire process of growth and sporulation may be studied, either with dry or immersion lens, in such preparations. J. G. Davis

F. Wrede. **Ueber das Prodigiosin, den roten Farbstoff des Bacillus prodigiosus.** (Prodigiosin, the red pigment of *B. prodigiosus.*) *Zts. f. Hyg.* **111**, 531, 1930. (*Bull. Inst. Pasteur*, **29**, xiv–xxxi, 704, July 1931.)

Prodigiosin is insoluble in water, soluble in fat solvents and more intense than fuchsin in colour. It is, however, turned yellow by sunlight and by acids. The empirical formula is $C_{20}H_{25}N_3O$. It forms well-defined salts, and is only reduced by hot sodium amalgam, but easily oxidised by permanganate. J. G. Davis

B. Blankov and Z. Gringot. **Unterschiede in der durch die Coli-Typhusgruppe hervorgerufenen Gärung unter aëroben und anaëroben Bedingungen.** (Differences in fermentation caused by the coli-typhoid group under aerobic and anaerobic conditions.) *Mikrobiol. Z.* **9**, 248–53, 1929.

The sugar-fermenting abilities of the coli-typhoid group under aerobic and anaerobic conditions have been tested by half covering growing slope cultures with vaseline oil. The results with 24 strains of coli were not uniform. The results with lactose, maltose, glucose and galactose media were the most consistent, the first two sugars only being fermented anaerobically and the last two equally well beneath and above the oil layer.

It is suggested that further development of this principle may lead to improved methods for species identification. J. G. Davis

L. H. Stickland. **The reduction of nitrates by Bact. coli.** *Biochem. J.* **25**, v, 1543–54, 1931.

B. coli quantitatively reduces nitrates to nitrites. Toluene does not affect the oxidation of formate and lactate either by oxygen or by nitrate, but cyanide inhibits this oxidation completely. Carbon monoxide is without effect.

In the presence of both oxygen and nitrate, lactate is oxidised chiefly by oxygen,

due to the "saturation concentrations" of the enzymes, hence a reduction of oxygen pressure leads to increased reduction of nitrate. J. G. Davis

G. S. Wilson. **The growth of Br. abortus (bovine type) in shake tubes.** *Brit. J. Exp. Path.* **12**, iii, 152–65, June 1931.

Bovine strains of *Br. abortus* when grown in agar shake cultures form a band about 2–5 mm. below the surface. Evidence is given that growth does not occur above this zone because of the loss of CO_2 into the air, or below because of the very low oxygen tension, both oxygen and CO_2 being necessary for growth.

The double zone phenomenon observed by Bang and Stribolt is explained by the alkali formation of the upper band, which, forming carbonates from the CO_2 present, removes the latter and so prevents growth for some little distance. J. G. Davis

Dairy Bacteriology.

Ministry of Health. **Memorandum on bovine tuberculosis in man with special reference to infection by milk.** *Reports on Public Health and Medical Subjects*, No. 63. (*J. State Med.* **39**, viii, 490–1, August 1931.)

The subject is dealt with under the following: the incidence of human tuberculosis in England and Wales; the proportion of cases attributable to bovine infection; the incidence of tuberculous disease in cattle with results of the tuberculin test, abattoir statistics, clinical cases detected by routine veterinary inspection and the proportion of cows suffering from tuberculosis; tuberculous milk, with a review of milk as a vehicle of infection, the proportion of cows yielding such milk and the proportion of milk so infected; the methods of controlling infection, at the source by eradication by slaughter, establishing tuberculosis-free herds, by immunisation with B.C.G., by routine examination of cattle and the bacteriological examination of milk, etc. The education of cow-keepers and the improved methods of cow-keeping are considered and the part played by the grading of milk in the campaign against tuberculosis. Pasteurisation is defined and the whole situation and subject briefly summarised.

H. E. Hasseltine and I. W. Knight. **Outbreak of undulant fever traced to infected milk supply.** *Pub. Health Rep.* **46**, xxxix, 2291–300, September 25, 1931.

Six cases of undulant fever occurred in a town of 5387 population. All drank raw milk from the same dairy. The cases were diagnosed by means of agglutination tests which ranged from 1 in 500 to 1 in 1280. The agglutination test was also applied to each animal in the herd, and of 42 tested, 24 gave positive reactions in various titres. *Br. melitensis* var. *abortus* was isolated from two samples of the milk. The cultures were isolated by incubation in an atmosphere of increased carbonic tension and gave the reaction characteristic of *Br. abortus* (Bang) when grown on media containing thionin, methyl violet, and basic fuchsin according to the method described by Huddleson. No further cases occurred after pasteurisation of the milk from this dairy had been adopted, notwithstanding that for a time the milk of some of the animals that shed *Brucella* in their milk continued to be sold. R. Stenhouse Williams

O. Bang, I. Jundell and H. Magnusson. **Nouvelles recherches sur la vaccination des bovidés contre la tuberculose suivant le procédé de Calmette et Guérin.** (New investigations on the vaccination of bovines by the method of Calmette and Guérin.) *Ann. Inst. Pasteur*, **47**, iv, 386–407, October 1931.

Calves from one herd were inoculated subcutaneously with from 1/50 to 1/100 mg. of a virulent bovine strain, some having previously been vaccinated by B.C.G. Of nine animals infected but not vaccinated, four died of generalised tuberculosis, two

became obviously tuberculous and three apparently healthy calves were found on autopsy to have extensive lesions. Of thirteen animals vaccinated previously to inoculation, six failed to develop lesions visible to the naked eye, three developed only insignificant lesions, one died, one was killed in a state of advanced tuberculosis and two were found on autopsy to have serious lesions. Thus while 100 per cent. of the unvaccinated calves developed tuberculosis lesions, only 54 per cent. of those vaccinated with B.C.G. did so. J. G. Davis

H. Alfonsus and O. Correll. **Nachweis von thermophilen Bakterien mittels der Reduktaseprobe bei 63° C. und ihre praktische Anwendung im Molkereibetrieb.** (Detection of thermophilic bacteria by means of the reductase test at 63° C. and its practical use in the dairy industry.) *Hildesheim Molk-Ztg.* **44**, 1237–9, 1930. (*Zbl. f. Bakt.* II, **83**, i–vii, 87, February 1931.)

To gain an insight into the relative thermophilic bacterial content of milk the methylene-blue reductase test at 63° C. is a simple and certain method. Pasteurisation apparatus used for a long time caused a great increase in thermophiles, and at the same time a sweet taste in the milk. Milk from the larger farms contained fewer thermophiles than holder-pasteurised milk from factories or raw milk from small holdings.

Rühmekorf. **Ueber die Bedeutung des Gesamtkeimgehaltes und des Colititers in der Milch. Der Coligehalt von Leipziger Milchsorten.** (Importance of total germ content and coli titre in milk. Coli content of various types of Leipzig milk.) *Milchw. Forsch.* **11**, vi, 600–14, April 1931.

This paper is an account of an investigation of the milk supply of Leipzig from the hygienic point of view. The total counts and coli figures are given for various types of milk. The lactose brom thymol blue trypaflavin agar of Klimmer was used for coli determinations in addition to the usual lactose broth media for detecting gas formation. The original concentration of 1 in 20,000 for the solid medium was found to permit the growth of micrococci, hence trypaflavin in a concentration of 1 in 10,000 was used. (*Milchw. Forsch.* **9**, 236.)

The coli colonies were somewhat smaller for this reason but still gave the typical colony form and yellowish colour. Usually the solid and liquid media results were correlated. A few milks gave gas without corresponding growths on the solid media and only very rarely was a solid medium "positive result" not confirmed by the gas tubes.

[For the solid medium test 1/10 c.c. of the milk is spread over dried trypaflavin lactose brom thymol blue agar in a Petri dish. Coli-aerogenes species give yellowish colonies, the aerogenes bacteria forming larger, opaque and slimy colonies, of a brighter yellow, and the paratyphoid group bluish forms. Either trypaflavin or rivanol may be used since these do not affect the indicator.] J. G. Davis

J. M. Rosell. **The bacteriology of chronic streptococcic mastitis.** *Cornell Vet.* **21**, iv, 317–33, October 1931.

K. D. Downham. **The examination of milk for the presence of tubercle bacilli—a comparison between the cell group method and a method for the concentration of tubercle bacilli in milk.** *Vet. Record*, **11**, xxix, 756–8, July 18, 1931.

The author states that in his experience the cell group method is more satisfactory than the concentration method of Douglas and Meanwell. The results of 31 samples of tuberculous milk are shown in which the first definite acid fast bacillus was found in a shorter time in 24 cases by the cell group method.

It is suggested that in cases of mixed udder infection, which include tuberculous mastitis, the concentration method may be the better.

The technique of smear preparation for examination by both methods is described.

J. McClemont

H. R. Curran. **The germicidal action of raw milk upon an organism of the sweet curdling type.** *J. Dairy Sci.* **14**, iii, 276–82, May 1931.

The effect of a sweet curdling Gram positive sporulating rod upon different milks has been studied. Raw mixed Holstein milk coagulated before raw mixed Jersey milk, but this difference was removed by sterilising the milk. This phenomenon was observed over a period of several months. Coagulation was delayed from 3½ to 6 hours in the case of the Jersey milk. The bacterial population at clotting was from 10 to 40 millions.

S. lactis was but little affected by the bactericidal substance, and did not appreciably influence the growth of the sweet curdler. Both organisms multiplied faster in the heated than in the raw (non-germicidal) milk. J. G. Davis

C. C. Walts. **The action of Penicillium roqueforti on butter.** *Arkansas Agr. Exp. Sta. Bull.* **257**, 51–2, 1930.

From this preliminary report of experiments in progress on the growth of various moulds and yeasts in butter and cream it appeared that *Penicillium roqueforti* actually grew in the butter when the moisture, salt and oxygen conditions were favourable. The butter acquired a mouldy flavour and an odour resembling Roquefort cheese. E. R. Hiscox

B. W. Hammer and J. A. Nelson. **Bacteriology of butter. II. A method for the microscopic examination of butter.** *Iowa Agr. Exp. Sta. Res. Bull.* **137**, February 1931.

The butter is melted and centrifuged in a separating funnel held in a special apparatus and the serum drawn off. 0·01 ml. is then spread over a given area of a slide as in the Breed method. The examination is then made according to the official methods. 10 ml. of butter give, on centrifuging for 1 min. at 1000 r.p.m., 1 to 1·4 ml. of serum according to the moisture content. Accordingly the serum count should be divided by from 11 to 7 to convert to the butter count. The percentage of bacteria left in the fat is about 0·1 per cent. J. G. Davis

B. W. Hammer and H. C. Olson. **Bacteriology of butter. III. A method for studying the contamination of churns.** *Iowa Agr. Exp. Sta. Res. Bull.* **141**, June 1931.

10 ml. of an agar medium is poured over a moistened area of the churn or other surface to be examined. After solidification the agar layer is removed and incubated in a Petri dish for 4 days at 21° C. Good photographs are given showing the types of "plate" obtained with churns of different condition. Modifications are discussed, *e.g.* the determination of yeasts and moulds on surfaces. J. G. Davis

W. G. Savage, F. C. Minett, *et al.* **Discussion on milk-borne streptococcus epidemics.** *Proc. Roy. Soc. Med.* **24**, xii, 1703–12, October 1931.

The literature of this subject was fully discussed and much new work reported. Reference was made to the recent Hove epidemic and its widespread nature in contrast to another, but smaller, outbreak in which contamination of the milk in transit was the probable cause (Wilkinson).

Savage, Minett and others agreed (i) that although streptococci were extremely common in milk the vast majority were of one or other of the common bovine types and harmless to man; (ii) that a number of epidemics of septic sore throat and scarlatina had been traced to actual infection of the cow with types not normally pro-

pagated in the bovine udder but implanted from a human source; such streptococci had not infrequently been found unassociated with any human disease; (iii) that contamination of milk in transit by streptococci was not a common source of outbreaks; the magnitude of the outbreaks and their continuous nature alone made this unlikely. Further, the growth of streptococci of human origin was inhibited in fresh cow's milk; (iv) that it had been shown that severe mastitis accompanied by the excretion of large numbers of organisms could be set up experimentally by means of a very small number of streptococci and that the condition often continued for weeks or months.

Minett stated that the streptococci of scarlatina and septic sore throat, being haemolytic, were not likely to be confused with non-haemolytic streptococci of bovine origin. Moreover, they were distinguished from the common haemolytic streptococci of bovines by their greater action on red cells, their inability to split hippurate to glycocoll and benzoic acid, their relatively low depression of the *p*H of lactose broth and their high virulence for mice and rabbits.

Stableforth reported that human streptococci were sharply distinguished from bovine strains of all types by their antigenic characters as demonstrable by precipitation and agglutination with immune sera.

The last two speakers also reported the isolation of a number of strains from severe cases of bovine mastitis which were indistinguishable as a group from *S. pyogenes* of direct human origin but unassociated with any known disease in man.

A. W. STABLEFORTH

CHEMISTRY (ORGANIC, INORGANIC AND PHYSICAL).

GENERAL.

O. L. OSBURN and C. H. WERKMAN. **Determination of organic acids. V. Application of partition method to quantitative determination of acetic, propionic and butyric acids in mixture.** *Ind. Eng. Chem.* (Anal. Ed.), **3**, iii, 264–5, July 1931.

The determined normality of the acid mixture must lie between 0·08 and 0·12. Equal volumes (25 c.c.) of the acid mixture are partitioned with two known volumes (30 and 60 c.c.) of isopropyl ether and the aqueous layers titrated. Percentage partition constants for these volumes having been worked out previously, three simultaneous equations are available from which the percentages of the three acids can be calculated. A nomogram for this purpose is included. W. L. DAVIES

S. ANSBACHER, R. E. REMINGTON and F. B. CULP. **Copper determination in organic matter.** *Ind. Eng. Chem.* (Anal. Ed.), **3**, iii, 314–7, July 1931.

A comparative study of the various micro-methods for the determination of copper in organic material is described. With the xanthate method the error is least when the amount of copper is 0·1 to 0·2 mg. while the Biazzo reagent is most reliable for 0·05 to 0·15 mg. and the carbamate for 0·05 mg. of copper. The chromotropic reagent is sensitive to 0·003 mg. and by colour comparison an amount of copper of this order can be determined within 0·0005 mg. W. L. DAVIES

G. M. MOIR. **The use of the quinhydrone electrode.** *Analyst*, **56**, 664, 445–8, July 1931.

The use of the quinhydrone electrode for the determination of hydrogen-ion concentrations has the advantage of rapidity and that semisolid material can be worked on. A description is given of a simple assembly of boiling tubes adaptable for the rapid determination of the *p*H of liquids given. The reference electrode consists of a solution 0·1 *N* with respect to both sodium acetate and acetic acid (*p*H 4·626). This is coupled by a saturated KCl bridge to a tube containing saturated KCl which in

turn is coupled to a tube containing the solution of unknown *p*H by a KCl-agar bridge. About 0·1 g. of quinhydrone is added to the electrode tubes (with immersed gold electrodes) and the E.M.F. of the cell determined by a potentiometer, the *p*H of the unknown liquid being calculated from the equation:

$$p\text{H} = 2{\cdot}03 + E/(0{\cdot}0577 + 0{\cdot}0002\,(t - 18)),$$

where E = E.M.F. and t is the temperature (° C.). Particulars are given for making, testing and cleaning the gold electrodes. W. L. DAVIES

J. G. DAVIS. **A simple inexpensive quinhydrone cell for rapid work.** *Analyst*, **56**, 664, 449–50, July 1931.

An apparatus is described for determining the *p*H of liquid or solid substances (*e.g.* cheese) without the aid of salt-agar bridges or glass taps. Each half cell consists of ½ in. pyrex tubing drawn out to a point and bent in the form of an **S**. The capillary tips dip in 3·5 *N* KCl. The reference electrode contains a solution of 9 parts 0·1 *N* KCl and 1 part 0·1 *N* HCl. Solids are ground with quinhydrone and either packed into a short piece of tubing, one end dipping into a crucible containing 3·5 *N* KCl while a gold electrode is inserted into the mush at the other end, or the mush is packed into a crucible and the tip of the reference electrode and a gold electrode inserted into it. Good results were obtained for well-buffered solids over a *p*H range of from 3 to 7. W. L. DAVIES

DAIRY CHEMISTRY.

E. LESNÉ, R. CLÉMENT and P. ZIZINE. **Sur la teneur en fer du lait de femme et du lait de certains mammifères (ânesse, chèvre, vache).** (Iron content of human milk and of that of certain mammals, ass, goat, cow.) *Le Lait*, **11**, 104, 355–8, April 1931.

The iron content was determined colorimetrically with alloxantin by the method of Zizine, using 500 c.c. of milk to avoid the error due to multiplication when a micro-estimation is performed. The milks of the four species contained approximately the same concentration of iron, viz. slightly less than 1 mg. per litre. There seems to be no justification for blaming a relatively low iron content as the cause of anaemia in animals fed on goat's milk. G. L. PESKETT

G. W. MONIER-WILLIAMS. **The nitrate test for the detection of added water in milk.** *Analyst*, **56**, 663, 397–8, June 1931.

The test for nitrate in milk can be made more sensitive if diphenylbenzidine be substituted for diphenylamine which has been recommended by earlier writers. The same blue compound (a quinone—imonium salt of diphenylbenzidine) is formed in both cases but the oxidation of diphenylamine at the expense of some of the nitrate is avoided. With diphenylbenzidine a good gradation of colour is obtained with amounts of nitrate ranging from 0·1 to 1 part per million. Marqueyrol and H. Muraour's method of preparing the reagent is described in detail. J. GOLDING

H. ILZHÖFER. **Ueber den Nachweis stattgehabter Erhitzung der Milch.** (Test for heated milk.) *Archiv f. Hyg.* **105**, vi, 319–21, April 1931.

A modified peroxidase test for heated milk is described. One part of a 1 per cent. solution of orthotolidin (Merk) in 10 per cent. acetic acid is added to five parts of 1 per cent. hydrogen peroxide. 0·5 c.c. of this mixture is then added to 5 c.c. of the milk serum (lead acetate method). The following colour reactions are claimed: raw milk—deep blue; holder-pasteurised milk—bright blue-green; milk held at 75° C. for ½ hour or 85° C. for 1–2 min.—no colour. J. G. DAVIS

R. Sasaki and E. Hiratsuka. **Phosphatides of cow's milk.** *Proc. Imp. Acad. Tokyo*, **7**, 99–100, 1931. (*Brit. Chem. Abst.* A, p. 862, July 1931.)

Methods are outlined for the isolation and separation of the phosphatides of dried milk. The fatty acid constituents are saturated, and the phosphatide content of milk containing 3·5 per cent. of fat is: myristolaurolecithin 0·003 per cent., and palmitolaurocephalin 0·007 per cent.

A. Schneck and C. Schlenz. **Ueber den Tyndalleffekt der Milch.** (The Tyndall effect in milk.) *Milchw. Forsch.* **11**, iv-v, 344–67, March 1931.

Experiments are described in the study of the turbidity of milk by the breaking up and absorption of light rays (Tyndall effect) by the disperse particles. The constituents mainly responsible for the effect are the fat in whole milk, the casein in skim milk, and the soluble proteins in the variously prepared sera. Significant differences were found for the effect of sera of pasteurised milk as against the sera of the same samples of milk in a raw state, but the sera of different samples of holder-pasteurised milk did not show significant variations. The application of this method to differentiate between raw and pasteurised milk needs further investigation.

W. L. Davies

L. A. Allen. **Observations on some alcohol-soluble proteins from milk products.** *Biochem. J.* **25**, iv, 1045–50, 1931.

Three proteins soluble in 80 per cent. ethyl alcohol, have been separated from Cheddar cheese (two) and from a peptic digest of casein (B.D.H. "Light White Casein"). The methods of separation and analysis are described. The proteins differed considerably in composition from casein and showed appreciable differences between themselves. The nitrogen content was lower (14·95–15·21 per cent.), but their main characteristic was a low phosphorus content (0·03–0·20 as against 0·82 per cent. in casein). The view that they are decomposition products of casein is discussed.

W. L. Davies

M. Polonovski and A. Lespagnol. **Le dosage des sucres du lait de femme par l'iode.** (Estimation of sugars of human milk by iodine.) *C.R. Soc. Biol.* **107**, xvii, 301–3, June 3, 1931.

The authors claim the presence of three different sugars in human milk, lactose, gynolactose and an unnamed third. The three differ in chemical and physical properties. Gynolactose can be estimated in the presence of lactose by taking advantage of its low reducing power against alkaline iodine solution (10 g. equivalent in reducing power to 3·4 g. of lactose). In the copper reduction (Bertrand) method also 10 g. is equivalent to 5 g. lactose. Differences in the rotatory power of human milk sera as against values calculated from analytical data can only be explained by the occurrence of a third sugar having the same reducing power as lactose but more dextrorotatory. Gynolactose accounts for 10–13 per cent. of the total sugars in human milk.

W. L. Davies

Many metals are soluble in milk. *Wisconsin Agr. Exp. Sta. Bull.* **420**, 71–2, February 1931.

Acidity of milk increases the solubility of zinc, tin, solder and aluminium and decreases the solubility of copper and its alloys and nickel. The important factor in the solution of the latter group being dissolved oxygen. Iron and aluminium are protected by dissolved oxygen. Increase of temperature increases the amount of all metals going into solution, reaching a maximum at about 160° F., irrespective of the acidity of the milk. Aluminium resists corrosion when no other metal is present, but the galvanic effects due to another metal being in contact with the same milk,

greatly increase the solubility. Copper alloys are more soluble than aluminium and the various stainless steels and should be used only in conditions where they show minimum solubility. Nickel is rapidly dissolved but has little effect on milk. Tin plating is not resistant to the corrosive effect of cleaning compounds and solder coated surfaces are appreciably soluble in milk. Chromium and nickel chromium steels resisted corrosion very well and resisted the attack of alkaline washing compounds. W. L. DAVIES

POPP. **The metals in the milk industry.** *Land. Vers.-Sta.* **111**, 271–91, 1931. (*Chem. Abst.* **25**, xii, 3093, June 20, 1931.)

The order of the decreasing resistance to corrosion of the five pure metals which were used is Ni, Cu, Fe, Zn and Al. The best alloy is a mixture of Fe, Ni and Cu.

H. T. GEBHARDT and H. H. SOMMER. **The solubility of metals in milk. I. The solubility of copper under various conditions.** *J. Dairy Sci.* **14**, v, 416–46, September 1931.

The solubility of copper in milk has been studied under careful experimental conditions. Higher acidity of milk decreased copper solubility. Oxygen and air increased and carbon dioxide decreased the solubility. Irrespective of length of time of exposure, maximum copper solubility took place at 70° C. Preheating milk decreased its dissolving effect. The rate of solution was found to be related to the oxidation-reduction potential. The interrelation of the various effects of the factors influencing the rate of copper solubility is discussed from the oxidation-reduction potential viewpoint. W. L. DAVIES

J. CSISZAR. **Dissolving effect of sour milk on lead plates.** *Kiserlet. Közlemenyek*, **32**, 495–501, 1929. (*Chem. Abst.* **25**, xii, 3093, June 20, 1931.)

The solution of lead from lead plates depends on the acidity of the milk, the time of exposure and the quality of the lead surface. Exposure of smooth bright lead plates for 36 hours to milk of 0·55 per cent. acidity caused no solution, but similar plates with a rough surface lost up to 0·24 mg. per square decimetre. Plates with smooth oxidised surfaces lost up to 0·14 mg. per dm.^{2} but rough oxidised plates in milk of 0·53 per cent. acidity lost 0·52 per dm.^{2} Increase in acidity increased the quantity of lead dissolved to a maximum, after which a decrease was observed. W. L. DAVIES

G. BERTRAND and P. SERBESCU. **Sur la toxicité de l'aluminium comparée à celle du fer, du nickel et d'autres métaux.** (The toxicity of aluminium compared with that of iron, nickel and other metals.) *Ann. Inst. Past.* **47**, iv, 451–7, October 1931.

The toxicities of some metals were compared by injecting 100 mg. per kg. live weight (dissolved as salts in distilled water) subcutaneously into the abdomen of the guinea-pig and the rabbit and noting the time of survival. The toxicities were found to be as follows (hours of survival) Cu 0·52; Ni 1·00; Cd 1·19; Co 2·17; Zn 3·01; Mn 7·14; Fe (ferric) 7·51; Al 8·34 and Fe (ferrous) 8·44. The author concludes that no danger is incurred by the use of utensils of aluminium for containing food. Copper and nickel should be avoided. J. G. DAVIS

G. TESTONI and W. CIUSA. **Determination of biacetyl in butter.** *Ann. Chim. applicata*, **21**, 147–50, 1931. (*Chem. Abst.* **25**, xii, 3094, June 20, 1931.)

As a possible method for identifying pure butter, a method has been developed for determining the biacetyl in butter. The biacetyl is separated from butter by steam distillation and treated in boiling solution with NH_2OH, and a few drops of 0·1 per cent. $NiSO_4$ solution and NH_4OH (concd.). A red precipitate of nickel dimethyl-

gloxime is formed. The precipitate may be weighed or estimated colorimetrically. Melted and pasteurised butter as well as margarines do not contain any biacetyl, whereas fresh butter contains about 0·0005 per cent. Its presence becomes a measure of the freshness of the sample of butter.

G. D. Elsdon, R. J. Taylor and P. Smith. **The Reichert, Polenske and Kirschner values of rancid butters and margarines.** *Analyst*, **56**, 665, 515–18, August 1931.

The Reichert value of butter is not appreciably changed when butter samples have been exposed for several weeks to the effects of light and air, although the free fatty acids might show considerable increase. Exposure to light causes a marked increase in the Polenske value. But with margarine fats, developing rancidity under similar conditions, a rise in the Reichert value is observed. The Kirschner value also increases whilst the Polenske value shows a variable change. If the butterfat present in a margarine is determined by the Reichert-Polenske + Kirschner process, care must be taken that the margarine is not rancid and that the values obtained for rancid mixtures be specially interpreted. W. L. Davies

M. Otte. **Der Wassergehalt der Butter. I. Eine Methode der Wassergehaltsbestimmung für Butter mit hohem Wassergehalt. II. Der Wassergehalt roher, ungekneteter Butter. III. Der Wassergehalt gekneteter Butter. IV. Ueber die Adhäsion zwischen Butterfett und Wasser resp. Butterfett und Salzlake unter verschiedenen Bedingungen. V. Ueber die Haltbarkeit der Butter, unter besonderer Berücksichtigung der Wasserverhältnisse in der Butter. VI. Untersuchungen zur Luftgehaltsbestimmung.** (Water content of butter. I. Method for estimating water content of butter with high water content. II. Water content of raw unworked butter. III. Water content of worked butter. IV. Adhesion of butterfat and water and butterfat and salt brine under various conditions. V. Keeping quality of butter, with special reference to the proportion of water in butter. VI. Determination of air content.) *Milchw. Forsch.* **11**, vi, 537–40; 541–50; 551–71; 572–6; 578–82; 583–9; April 1931.

I. In determining the moisture content of unworked butter the sampling and preparation of the sample are important. It is advisable to emulsify thoroughly, preferably adding an emulsifying agent (in this case, Palsgard Emulsionsol). Samples of about 2 ml. can then be pipetted out and the moisture determined, by drying in a vacuum oven at 100° C. (Boysen method).

II. The moisture content of unworked washed butter depends primarily on the total area of the fat globules and on the temperature conditions which determine the adsorption of water on the fat. The influence of churning temperature is counteracted by washing. If the cream acidity is sufficiently high to cause protein coagulation the increase in surface thereby causes an increase in moisture content. The previous treatment of the cream has little effect, but washing with salt water diminishes the moisture content, depending on the salt content of the water.

III. The working of butter decreases the moisture content to a minimum value, after reaching which, further working causes a slight increase of moisture. This "minimum moisture content" is lowered by long cooling of the cream, but cream acidity has very little effect. Increasing the speed of the butter-worker increases the value, but pasteurisation of the cream, length of churning time and speed of the churn do not affect the value beyond the usual limits of variation. The degree of churning of the cream in determining the grain of the butter has an important effect on the moisture content, over-churning causing a reduction.

IV. The conditions in the churning of butter which govern the adhering of moisture to butterfat are: the temperature of the wash water, the type of fat, summer

butterfat causing more moisture to adhere to it than winter fat; the method of cooling cream, in that sudden chilling of cream to a low temperature gives a butter of higher moisture content than cream kept at a moderately low temperature for a considerable length of time; on the quantity of dissolved salt in the brine, in that less adhering of water to fat occurs in brine than in water.

V. Storage of butter at − 18° C. preserved well the texture of butter. There was no difference in the texture of spring and summer butter, of unsalted, slightly or heavily salted butter under such storage conditions. Variations in moisture content in butter did not influence texture during storage and there was no effect due to high air content in butter.

VI. An accurate method is described for determining the air content of butter depending on the fact that air is the only component of butter liable to change in volume under pressure. The air content is of no practical importance in butter.

W. L. Davies

L. H. Lampitt and N. D. Sylvester. **Action of air and light, particularly on butter.** *Chimie et industrie*, Spec. No., pp. 642–7, March 1931. (*Chem. Abst.* **25**, xiv, 3737, July 20, 1931.)

It is considered that the taste of rancid fat is the resultant of two different tastes, one due to structural changes in the glycerol portion of the molecule, and the other caused by changes in the constituents producing the original taste, and it is thought possible that one factor can come into play independently of the other. Lampitt and Sylvester wish to restrict the term "rancidity" to include only the phenomena resulting from changes in the glycerol radical of a fat or oil, and to introduce the expression "tallow taste" to designate the taste due to the changes taking place in the constituents producing the original taste. The Kreiss test (which does not lend itself to a quantitative study of the changes occurring in fats) does not give a true indication of the rancidity (considered from an organoleptic standpoint). The Issoglio test is useful, not as an indication of rancidity or tallow taste but as a method of measuring the initial changes taking place when a fat deteriorates under the effects of air and of ultra-violet rays, and it can give a quantitative measure of these changes before the stage of positive Kreiss tests has been reached. Experimental results obtained by irradiation (in the presence and absence of air) of Et_2O and petroleum ether solutions of butterfat support these views.

G. Genin. **Constitution physico-chimique de la poudre de lait desséché. Les matières grasses de la poudre de lait desséché par pulvérisation.** (Physico-chemical constitution of dried milk powder. The fat of spray-dried milk powder.) *Le Lait*, **11**, 106, 589–97, June 1931.

In a review of the subject the author points out that comparatively little work has been done on the physico-chemical properties of milk powder and that probably the contradictory results obtained by different workers as to the effects of storage on keeping quality and solubility are due to the fact that they have compared powders with very different physical structures.

Since it is difficult to bring colouring matters and solvents into contact with the fat contained in milk powder, it is assumed that the fat is protected by a film of protein. Lampitt and Bushill have therefore defined "free fat" as that portion of the fat which can be extracted by solvents such as carbon disulphide under standard conditions.

Figures are given which show that for eight samples of "Milkal" spray-dried milk the percentage of total fat which is in the free condition varies from 3·3 to 14·2, while for five samples of roller-dried milk the percentage varies from 91·6 to 95·8. The

author concludes that such a considerable difference between the two types is due to the fact that, in spray-drying, the milk is actually dried in the form of particles whereas, in roller-drying, it is dried as a continuous film and pulverised afterwards. The size of the particles is a factor influencing the free fat content of spray-dried milk; thus crushing results in an increase because of the greater total surface.

In spray-dried milk there is a critical moisture content at which the fat becomes free and is extracted by solvents. For powder with a fat content of 27–8 per cent., this critical moisture content is 8·6–9·2 per cent. L. A. ALLEN

C. L. HINTON and T. MACARA. **The determination of laevulose in sweetened condensed milk.** *Analyst*, **56**, 662, 286–92, May 1931.

Laevulose may be present in sweetened condensed milk either as a product of the breaking down of sucrose or from invert sugar added. The method of its estimation depends on its copper reduction capacity after the preliminary oxidation of aldose sugars (glucose and lactose) by alkaline iodine. Excess of iodine used for oxidation of aldose sugars is quantitatively removed with sodium sulphite solution, while the copper reduction by laevulose is carried out by using the citrate-carbonate reagent of Luff (similar to Benedict's quantitative copper reagent). Certain standard technique is essential and factors for calculation are supplied for which the original paper should be consulted. W. L. DAVIES

BIOCHEMISTRY.

J. WADDELL and H. STEENBOCK. **Vitamin E in iron-treated dry rations.** *J. Nutrition*, **4**, i, 79–93, May 1931.

A mixed stock ration (Waddell and Steenbock, *J. Biol. Chem.* **80**, 431, 1928), treated with an ether solution of ferric chloride (1 part of iron salt to 99 parts of the diet) produces sterility in male rats reared on it. It also induces 100 per cent. sterility in female rats. There is no first litter fertility. The iron treatment brings about the formation of a substance which actively opposes vitamin E. It can be extracted from the iron treated ration with ether. It may be an anti-vitamin or possibly possess pro-oxygenic properties favouring the destruction of vitamin E. S. K. KON

D. L. HUSSEMANN and R. A. HETLER. **The vitamin B and G requirements of lactation.** *J. Nutrition*, **4**, i, 127–40, May 1931.

Either vitamin B or G in quantities above and added to the maintenance level of the vitamin B complex are required for lactation. It is suggested that vitamin G is the most important for successful lactation.

(Vitamins B and G are called B_1 and B_2 in the terminology adopted in England.) S. K. KON

G. C. SUPPLEE and O. D. DOW. **The effect of ultra-violet irradiation on the anti-scorbutic vitamin of liquid and dry milk.** *Amer. J. Dis. Child.* **41**, 1353–62, 1931. (*Bull. Hyg.* **6**, x, 769, October 1931.)

Irradiation of dry milk caused no destruction of the vitamin C contained therein, irradiation of liquid milk caused slight destruction of vitamin C, but the amount remaining in the milk was not reduced below the level frequently found in untreated milk. Irradiation in both cases was of sufficient duration and intensity to impart marked antirachitic properties to the milk. R. G. BOOTH

K. SCHLEER. **The prophylaxis of rickets by means of irradiated milk.** *J. State Med.* **39**, x, 607–9, October 1931.

Milk irradiated by the Scholl system will cure advanced rickets in four to eight weeks when fed (to children) at the rate of half a litre daily. The milk is unaltered in

flavour or smell and is readily taken. Prophylaxis is accomplished by 300–400 c.c. of the milk daily. Half a litre a day drunk by the nursing mothers confers absolute freedom from rickets on her child. R. G. Booth

B. H. Thomas and F. L. MacLeod. **Increasing the vitamin D potency of cow's milk by the daily feeding of irradiated yeast or irradiated ergosterol.** *Science*, **73**, 618, 1931. (*Amer. J. Publ. Health*, **21**, viii, 951, August 1931.)

In this experiment varying quantities of vitamin D as irradiated yeast and irradiated ergosterol were fed to dairy cattle, and the effect of this supplementary feeding on the vitamin D content of the butterfat was investigated.

The cows were 21 in number, divided into 7 groups of three each, and were fed on a basal ration of alfalfa meal, corn silage, beet pulp and grain mixture. The pre-experimental period lasted 3 months and the experimental supplements were fed for a period of 4 weeks.

The results obtained are summarised in the following table where the vitamin D potency of the butterfat of the control group of cows is taken to be one arbitrary unit, the potency in rat units per gram not being stated:

Group	Supplement	Vitamin D, No. of rat units of supplement given *per diem*	Vitamin D, potency of butterfat
I, Control	—	—	1
II	Irradiated yeast	10,000	2
III	,,	30,000	8
IV	,,	60,000	16
V	Irradiated ergosterol in corn oil	15,000	2
VI	,, ,,	45,000	4
VII	,, ,,	135,000	16

The fact that the vitamin D potency of milk can be easily and cheaply increased to this extent is important, especially in so far as it increases the assimilation of the calcium and phosphorus of the milk. R. G. Booth

E. G. Brown. **Report of Committee on Milk, Conference of State and Provincial Health Authorities of North America.** *Publ. Health Rep.* **46**, xxv, 1455–60, June 19, 1931.

The Committee recommends the acceptance and approval by the Conference of State and Provincial Health Authorities of the entire Report of the Committee on Milk Production and Control, of the White House Conference on Child Health and Protection (cf. abst. *J. Dairy Res.* **3**, i, 175). The Committee advocate that milk consumption should be increased rather than reduced, even when it becomes necessary to readjust the family budget. Two articles, one by Scott and Erf (cf. *Jersey Bulletin*, February 11, 1931), the other by Mattick and Golding (*The Lancet*, p. 662, March 21, 1931), postulating a higher nutritional value for raw milk as compared with pasteurised milk, are discussed, and the experimental results are considered to be inconclusive and to afford no proof that raw milk possesses a dietary superiority over pasteurised milk.

Four other articles expressing the view that the nutritional value of milk is not impaired in the course of pasteurisation are cited. The committee urges the (U.S.) Public Health Service to undertake a study to determine the present experience with reference to the feeding of infants with raw and pasteurised milk.

(One of the four articles cited by the committee—the Lanarkshire Report—has recently been subjected to statistical analysis by Bartlett and Fisher, and Bartlett (*Nature*, No. 3207, 591, April 18, 1931 and *J. Min. Agric.* **38**, i, 60–4, April 1931), and the results are believed by these authors to be in favour of raw milk.)

S. K. Kon

J. WADDELL with the co-operation of E. VAN DONK. **Male sterility on milk diets.** *J. Nutrition*, **4**, i, 67–77, May 1931.

Total sterility in male rats has been observed on a diet of cow's whole milk supplemented with small amounts of iron and copper salts. This sterility is characterised by the complete disappearance of the germinal epithelium, great loss in amount of testicular tissue, and pronounced oedema. The view is expressed that this sterility is not due to lack of vitamin E. The injury to the testis is less marked in animals receiving a diet of whole milk and copper only and is apparently intensified by some action of the iron salt (ferric chloride). S. K. KON

J. WADDELL, H. STEENBOCK and E. B. HART. **Growth and reproduction on milk diets.** *J. Nutrition*, **4**, i, 53–65, May 1931.

On a diet of whole milk and copper a chronic anaemia ascribed to the low iron intake was observed. Growth and reproduction were below normal. Many of the young born were not nursed, and those that were suckled grew more slowly than young from the stock colony, being from 10 to 12 g. underweight at weaning. On a diet of whole milk, iron and copper, no marked anaemia was observed (haemoglobin values 12 g. per cent.) but growth and reproduction were still subnormal. The rearing of young was poor, due to poor mammary secretion. It is suggested that subnormal growth may be due to a low intake of calories in the liquid diet. No specific evidence of low vitamin B was observed. By means of daily vaginal smears late sexual maturity and very poor ovulation were detected in the females. Some of them never mated. Preliminary experiments indicate that the addition of small amounts of either manganese or iodine, or both, greatly improves the ovulation rhythm in females on the milk, copper, and iron diet. No evidence of lack of vitamin E was noted in females on these milk diets. S. K. KON

E. W. SCHWARTZE, F. J. MURPHY and G. J. COX. **The effect of pasteurisation upon the vitamin C content of milk in the presence of certain metals.** *J. Nutrition*, **4**, ii, 211–25, July 1931.

Certified milk (U.S.) about 12 hours old was pasteurised in a specially constructed laboratory apparatus in three pasteurisers constructed in aluminium, tinned copper and naked copper respectively. After a preliminary preheating period of about 10 min. the milk was kept for 30 min. at a temperature ranging from 58·5 to 60·5° C. It was then fed to guinea-pigs receiving a scurvy-producing diet. The experiment took place between the middle of May and the middle of September. The results indicate a destruction of 20–40 per cent. vitamin C in the aluminium pasteuriser, a slightly higher loss in the tinned copper one and at least 80° per cent. destruction when naked copper was used. Aluminium is recommended as a satisfactory material for the construction of dairy equipment. S. K. KON

S. V. TELFER. **Mineral metabolism in infancy. III. The substitution of cow's milk for human milk in infant feeding.** *Glasgow Med. J.* **114**, 265–84, 1930.

The diet of five infants (aged 7 to 11 months) was changed from human to cow's milk. The change resulted in

(i) Reduced mineral retention as a whole (percentage of the intake);
(ii) Reduced retention of iron;
(iii) Increased retention of bone-forming elements; and
(iv) Increased faecal output as regards bulk and mineral and fat content.

These results are explained on the basis of the exceedingly large mineral content of cow's milk, except in iron, and the fact that effective mineral absorption depends

on the maintenance of the elements in soluble form by the acid agency of the upper part of the alimentary tract.

In early infancy the acidity of the tract is very low, and a high degree of absorption can be attained with human milk on account of its low mineral content. The use of cow's milk is inadmissible as the large excess of minerals results in formation and excretion of large amounts of insoluble soaps which impair fat absorption and mineral absorption as a whole. The large excess of bone-forming elements in cow's milk allows greater retention of these, but the percentage retention of the intake of these and all elements is reduced. Thus in the case of iron, the concentration of which is no higher in cow's than in human milk, the retention is diminished to dangerously low levels.

From the first year onwards there is risk of mineral deficiency if breast feeding be continued. Cow's milk, on the other hand, is well adapted to provide for the increased mineral requirements, but needs supplementing with iron. The value of cow's milk in the nutrition of school children is discussed briefly. G. L. Peskett

W. B. Nevens and D. D. Shaw. **Effect of different plant processes (e.g. pasteurisation, condensing, drying) on the nutritive value of milk.** *Illinois Agr. Exp. Sta. Ann. Rep.* 1929/30, pp. 127–33.

The object of these experiments was to find out if the treatment of milk by the specified processes would add enough copper or iron to affect its nutritive value.

The failure of rats to become anaemic on a diet of milk is attributed to the use of iron cages and to other metallic contamination. When filter paper was used in glass jars this also supplied iron. When rats were kept in cages made only of wood and glass with glass rods for flooring they developed anaemia. Restricted intake of milk caused the anaemia to develop more slowly. J. Golding

K. Ried. **Untersuchungen über den Katalasegehalt der Kuhmilch.** (Investigations on the catalase content of cow's milk.) *Milchw. Forsch.* **11**, vi, 590–9, April 1931.

Determination of the catalase content of milk has been suggested as a means of diagnosing udder disease in the early stages. The author found that even aseptically drawn milk gave a positive catalase reaction. Various factors influenced the catalase content, *e.g.* first-drawn milk usually contained less than the last-drawn; the catalase content decreased during the first 8 days after the change from dry fodder to green stuff; and vice versa, the milk of pregnant cows showed a high catalase value during the last weeks before calving. Further, the catalase content of the milk of sick cows may be either high or low. Its determination will therefore be of little value for diagnostic purposes. E. R. Hiscox

K. A. Forster. **Vitamingehalt der Sauermilchpräparate Yoghurt, Kefir und Saya.** (The vitamin content of the sour milk products, yoghurt, kefir and saya.) *Biochem. Z.* **236**, iv–vi, 276–97, 1931.

Yoghurt is richer in vitamins A and D but contains less vitamin B and C than an equal weight of milk used for its preparation. The increase of the fat-soluble vitamins is only apparent, caused by a concentration of milk in the course of the manufacture of yoghurt. Kefir contains less vitamins A, C and D than the original milk. There is no significant difference in the vitamin B content. Saya is twice as rich in vitamin A as the original milk and contains 1/3 more of vitamin C. Vitamins B and D remain unchanged. It is suggested that vitamins A and C are actually synthesised by bacteria in the course of fermentation. S. K. Kon

M. Lundborg. **Zur Kenntnis des A-Vitamins im Butterfat.** (The study of vitamin A in butterfat.) *Biochem. Z.* **235**, i–iii, 1–13, May 30, 1931.

Higher values are obtained for the carotin content of butterfat when they are derived from the antimony trichloride reaction than when the carotin is estimated by direct colorimetric comparison of solutions of butterfat in petroleum ether with solutions comparing known amounts of pure carotin. The difference is due to the presence in butterfat of a colourless carotenoid substance—"Carotenoid X"—giving the antimony trichloride reaction and probably identical with vitamin A. S. K. Kon

B. G. E. Morgan and K. H. Coward. **Estimation of vitamin A in butter.** *Lancet*, No. 5640, 758–9, October 3, 1931.

The method described by Coward, Key, Dyer and Morgan (*Biochem. J.* **24**, 1952, 1930, and **25**, 551, 1931) and by Coward, Dyer, Morton and Gaddum (*Biochem. J.* **25**, 1102, 1931), for the estimation of vitamin A in cod-liver oils is shown to give consistent results also in the estimation of vitamin A in butter and margarine.

S. K. Kon

H. Steenbock and A. M. Wirick. **Fat-soluble vitamins. XXXIII. The determination of vitamin A and its stability in butterfat to ultra-violet radiations.** *J. Dairy Sci.* **14**, iii, 229–49, May 1931.

Vitamin A was determined by the method of Steenbock and Coward (*J. Biol. Chem.* **72**, 765, 1927), and it was found that it is developed by ultra-violet treatment even when the irradiation was carried out in an atmosphere of carbon dioxide, nitrogen or hydrogen, though the presence of these gases apparently retarded somewhat the destruction. This was found to be proportional to the time of exposure and inversely proportional to the thickness of the layer of butter exposed to ultra-violet irradiation. The irradiation necessary to secure maximum antirachitic activation of the butter was found to cause some destruction of vitamin A. Butterfat antirachitically activated was found to have retained most of its vitamin A even after storage for seven months at room temperature. S. K. Kon

R. W. Jackson. **The effect of mineral oil administration upon the nutritional economy of fat-soluble vitamins. I. Studies with the vitamin A of butterfat.** *J. Nutrition*, **4**, ii, 171–84, July 1931.

The author was able to confirm the findings of Dutcher, Ely and Honeywell (*Proc. Soc. Exp. Biol. and Med.* **24**, 953, 1927), that mineral oil causes a considerable loss of vitamin A in butterfat when mixed with the latter prior to ingestion, as measured on the rat. However, the administration of the mineral oil separately from the butterfat under conditions approximating those customary in human practice, results in only a very slight loss of vitamin A to the animal organism. S. K. Kon

A. Scheunert and E. Wagner. **Ueber das Verhalten des Vitamin-A-Gehaltes der Butter bei verschiedener Erhitzungsweise.** (The behaviour of the Vitamin A content of butter when heated in different ways.) *Biochem. Z.* **236**, i–iii, 29–34, June 25, 1931.

No perceptible decrease in the vitamin A content of butter could be detected by the rat growth method after it had been used in the customary way for baking, roasting or browning. A marked loss of activity is caused by heating the butter for ½–1 hr. in open pans at a temperature of 160–200° C. S. K. Kon

PHYSIOLOGY.

C. M. McCay and L. A. Maynard. **The interrelationship between the dietary fat and the phosphorus distribution in the blood of lactating cows.** *J. Biol. Chem.* **92**, ii, 273–80, July 1931.

Cows were fed for 5-week periods at three levels of fat intake, approximately 1, 3 and 7 per cent. The caloric intake was equalised by substitution of starch for the fat that was removed. Milk and fat yields were lower during the periods of lowered fat intake, as were the phospholipids and total phosphorus of the blood plasma, but not of the erythrocytes. It appears that the body cannot synthesise sufficient fat to permit the milk secretion that can occur when larger quantities of fat are given in the ration, though it should be noted that the change in the blood was gradual after changing the fat intake. G. L. Peskett

A. Leroy and J. Marcq. **Le taux butyreux sanguin des reproducteurs bovins et ses rapports éventuels avec la valeur d'élevage (suite).** (The butyric blood number of breeding cattle and its eventual bearing on the value of breeding (contd.).) *Le Lait*, **11**, 103, 234–44, March 1931; 104, 259–69, April 1931.

In continuation of previous work it was found that the concentration of fatty acids of the blood show changes similar to the fat yield during the course of a complete lactation, falling to a minimum in the second or third month and then rising. Variations were also noted in relation to age and sex. With some exceptions (notably Jersey cows) the percentage of butterfat in the milk corresponded to the concentration of fatty acids in the blood ($r = + 0{\cdot}558 \pm 0{\cdot}08$), and to the concentration of total lipoids in the blood ($r = + 0{\cdot}601 \pm 0{\cdot}08$). In the case of a number of animals, all the progeny of the same sire, it was claimed that the fatty acids concentration in the blood placed them in the same order as the mean butter fat percentage of their respective dam's milk.

(The number of observations made, is, as the authors admit, almost too small to justify statistical analysis. From the physiological point of view it is doubtful whether any significance can be attached to the correlation which was found between the average butterfat percentage of one or more complete lactations and the average blood fatty acid percentage of one or two random samples.)

The last paper of the series records the experimental data of the individual cows that were studied. G. L. Peskett

L. B. Winter. **The metabolism of lactose. I. The occurrence of lactose in urine.** *J. Physiol.* **71**, iv, 341–55, April 1931.

This paper opens with a good discussion of previous work on the metabolism of lactose. In it are recorded 29 cases (human) of whom 16 showed lactosuria in the first few days of lactation though their urine had been sugar-free on the day after parturition.

Administration of 50 g. of lactose by mouth causes very little to appear in the urine unchanged; its first effect is appearance of glucose or galactose or both. On the other hand, injection of lactose into rabbits causes excretion of it in the urine—in some cases in amounts greater than that injected. G. L. Peskett

J. Allardyce, R. H. Fleming, F. L. Fowler and R. H. Clark. **Blood normals for cattle; some pathological values.** *Canadian J. Res.* **3**, ii, 120–4, August 1930.

This paper records analytical results for ten constituents of the blood from twenty-two normal cows. No significant variations were apparent except a marked effect of diet on the cholesterol content of blood. In nine animals suffering from various diseases the only notable variations occurred in a case of pyelonephritis, in which the conditions of the renal system might readily account for them. G. L. Peskett

H. S. Mitchell and L. Miller. **The effect of age, pregnancy and lactation on the haemoglobin of the albino rat.** *Amer. J. Physiol.* **98**, ii, 311–17, September 1931.

The haemoglobin content of the blood of young rats receiving a stock colony diet increased from 8·9 to 15·6 g. per 100 c.c. during the period from 23 to 50 days of age. The average for adult rats (57 to 578 days of age) was 16·2 g. per 100 c.c. No significant difference was found between the sexes in this respect. The development of an anaemia, preceding parturition and progressing for three days following with a subsequent spontaneous recovery has been observed in at least 92 per cent. of 145 pregnancies studied. (The maximal drop was to about 13·0 g. of haemoglobin per 100 c.c. of blood.) Neither the addition to the diet of a mineral mixture containing iron, copper and manganese sulphates nor the inclusion of 0·59 daily of a yeast extract rich in vitamins B_1 and B_2 exerted any apparent influence in preventing the anaemia of pregnancy. S. K. Kon

L. A. Maynard, E. S. Harrison and C. M. McCay. **The changes in the total fatty acids, phospholipid fatty acids and cholesterol of the blood during the lactation cycle.** *J. Biol. Chem.* **92**, ii, 263–72, July 1931.

The fatty constituents of the blood were studied during the dry period and a succeeding lactation. Following parturition a rapid rise occurred, succeeded by a gradual fall during lactation to the previous low level of the dry period. These changes were noted in total fatty acids, phospholipid fatty acids and cholesterol, the values for each running closely parallel, and approximately following the changes in milk yield, but not milk fat percentage. Although the values may be influenced by food intake, the same changes occurred at a constant level of intake and appeared to be definitely associated with lactation. The study was made on 4 cows, one of which suffered an acute attack of mastitis during the experiment; the changes at parturition were also noted in the case of two other cows. G. L. Peskett

L. B. Winter. **Cellobiose in relation to the mammary gland.** *J. Physiol.* **73**, iv, 6*P*, December 1931.

In order to test the possibility of lactose being formed from a β-glucoside, cellobiose was incubated with various extracts of mammary tissue. Under the conditions of experiment no lactose formation could be detected, nor could any evidence of utilisation of cellobiose be obtained when it was injected into lactating rabbits.
G. L. Peskett

O. Svanberg. **Enzymatische Versuche mit Milchdrüsen.** (Enzymatic research with milk glands.) *Hoppe-Seylers Z.* **188**, 207–18, 1930. (*Milchw. Forsch.* **11**, iv–v, Ref. 73, March 1931.)

The author records experiments in which an attempt was made to imitate *in vitro* the sugar metabolism of living udder tissue. Preparations of washed and ground mammary gland showed, however, practically no ability to form lactose from glucose, galactose or mixtures of the two. On the contrary, the sugars used, which also included lactose, were almost completely broken down in 21 cases out of 24, the chief end-product being lactic acid. The effect of various antiseptics was investigated.

Negligible quantities of sugars other than lactose were found to be present in milk, as judged by reduction with Barfoed's reagent. G. L. Peskett

W. B. Cannon and E. M. Bright. **A belated effect of sympathectomy on lactation.** *Amer. J. Physiol.* **97**, ii, 319–21, May 1931.

Removal of the sympathetic nervous system in the dog or cat may cause disturbance of lactation (shortage or absence of secretion) but does not interfere with other reproductive processes. G. L. Peskett

F. R. Krzywanek and H. Brüggemann. **Zum Studium der Milchsekretion. II. Der Druckablauf in allen 4 Zitzen des Euters während des Milchentzuges.** (Study of milk secretion. II. Release of pressure in all four teats of the udder during the withdrawal of milk.) *Milchw. Forsch.* **11**, iv–v, 371–8, March 1931.

The authors show that the pressure in the individual quarters of the udder falls during milking of those quarters, but it stays relatively constant in the remaining (full) quarters. The conclusion suggested is that there is little, if any, secretion of milk during actual milking and that the udder can accommodate its contained milk at a certain pressure by tonic control of its contractile elements whatever may be its degree of fulness.

G. L. Peskett

J. W. Gowen and E. R. Tobey. **Studies on milk secretion. The influence of inanition.** *J. Gen. Physiol.* **15**, i, 45–66, September 1931.

The following changes occurred when cows were deprived of food but allowed free access to water: rapid fall in yield and lactose percentage of the milk, with a rise in total solids, butterfat and ash percentages (the calcium percentage rising out of proportion to other elements of the ash, while phosphorus showed little change); a slight fall in blood sugar and a definite fall in blood calcium percentages in the majority of cases. Administration of parathyroid extract caused a slight rise in the blood calcium and sugar percentages whether the animal received a full ration or not. In all cases there was a tendency for the osmotic pressure of the milk to rise.

It was claimed that "all the changes in milk composition during starvation can be directly related to the simultaneous changes in the blood," *e.g.* fall in blood sugar causing fall in lactose with a compensatory rise in salts of the milk; rise in osmotic pressure of both blood and milk; independent movement of butterfat as contrasted with the other solids.

(Careful survey of the authors' charts suggests that they hardly warrant the conclusions drawn. Changes in composition of blood and milk seem to have been overemphasised in some places and disregarded in others, especially in the case of lactose and blood sugar percentages and fat and total-solids percentages. Thus reading off values from the curves, decrease in lactose 0·5 per cent. occurred with drop in blood sugar 0·015 per cent. in three instances, 0·007 per cent. in one instance and 0·002 per cent. in another. Similarly a fall of 1·5 per cent. in lactose occurred with a drop in blood sugar of only 0·01 per cent., and a rise of lactose only 0·2 per cent. corresponding to rise in blood sugar 0·02 per cent. As regards fat percentages of the milk, these are by no means independent, since in every case their curve follows that of the total solids per cent. Moreover the figures for osmotic pressures of milk were calculated from protein, ash and lactose percentages (the distribution of ash elements was assumed to be normal although the Ca and P, the only elements analysed, showed independent movement), and parallel changes in blood were assumed to occur in the cows solely on the basis of previous work of other authors on dogs and rabbits).

G. L. Peskett

J. W. Gowen and E. R. Tobey. **On the mechanism of milk secretion. The influence of insulin and phloridzin.** *J. Gen. Physiol.* **15**, i, 67–85, September 1931.

The effects of insulin treatment on milk secretion in the case of starved and unstarved cows were similar to those reported in the previous paper (though more immediate and severe in character) except that the calcium and phosphorus percentages of the milk rose with the ash. In one case a cow was subjected to prolonged insulin shock, the milk solids rose to 37 per cent. (butterfat to 24·8 per cent.) while the yield dropped almost to zero. Subsequently this animal's production returned to 75 per cent. of the normal. In phloridzin treatment combined with starvation there

was a drop in yield and lactose per cent., a rise in ash per cent. and a rise followed by return to normal in total solids and fat per cent.

The authors consider that these experiments support the conclusions of the previous paper. They interpret the results as showing that in milk secretion a balance is maintained between the osmotic pressure of the milk and of the blood, modification of the blood producing direct and predictable modification of the milk secreted, especially as regards sugar.

(Interpretation of the results is not easy. The significance of the composition of the milk seems to be very doubtful at the very low level of yield reached during the experiments. No observation was made of the blood osmotic pressures, and it is hard to explain the wider variation of milk osmotic pressures which occurred in these experiments in which insulin treatment was the only additional factor. Although it is true that the lactose percentage follows the general trend of the blood sugar percentage, in several instances a definite rise of the former occurred while the values of the latter were falling. Moreover it cannot be said, as the authors claim, that the butterfat percentage is more or less independent of the other constituents, for in every case its curve follows closely that of the total solids per cent.) G. L. Peskett

DAIRY HUSBANDRY.

E. W. Fenton and E. D. S. Robertson. **Poisonous and milk tainting plants. Symptoms and first aid to stock.** *Edinburgh and E. Scotland Coll. Agric.* N.S. No. 4, 1931.

The pamphlet aims at providing owners of stock with information likely to help them in cases of poisoning of animals or milk taints caused by plants. There are two distinct sections in the booklet, one "Botanical" and the other "Veterinary." The former gives the botanical features of a number of herbs and the latter deals with the symptoms resulting from the various poisonous plants and first aid treatment.

It is doubtful if the booklet bridges the gulf between the scientist and the practical man in the best possible manner, and no very concise statement is given which might help a farmer to decide whether his sick animals or his tainted milk are due to plants or to some other cause.

The two sections appear more convenient to the writers than to the reader, while the choice of sub-headings and terminology is more closely allied to the scientific text books than to the vocabulary of the average farmer. S. Bartlett

W. E. Agar. **Heredity and milking function. An analysis of the inheritance of milk and butterfat production in Red Poll cattle.** *J. Dept. Agric. Victoria*, **24**, i, 1–9, January 1926.

In this article, based chiefly on records of the Red Poll herd of the Victoria State Research Farm, it is concluded that daughters' production is definitely influenced by, but not absolutely determined by the mother. A method of comparing the transmitting qualities of bulls for production is put forward. A brief outline of the method is given below, using fat percentage as a typical inheritable factor:

1. The correlation coefficient between the fat percentage of dams and offspring in the herd records was found to be 0·332. The regression coefficient of the fat percentage production of daughters relative to their dams was 0·291. The average production of dams was 4·40 per cent. fat and of daughters 4·30 per cent. fat.

If a single dam's production is 0·40 per cent. fat above the general average (*i.e.* 4·80 per cent. fat) her daughters on the average may be expected to yield $0{\cdot}40 \times 0{\cdot}291$ above the average of all daughters, *i.e.* $4{\cdot}30 + (0{\cdot}40 \times 0{\cdot}291) = 4{\cdot}42$.

2. Using the above method, the "expected test" of all the daughters of a single bull can be calculated, and it is assumed that this makes an average allowance for the influence of dams on their offspring.

3. The extent to which daughters of a bull exceed or fall below their "expected test" is used as an indication of the transmitting ability of a bull for production.

S. BARTLETT

K. W. D. CAMPBELL. **Possible influence of the milkers on the quantity and quality of milk of the individual cow.** *Agricultural Progress*, **8**, 114–20, 1931.

Cows milked thrice daily at equal intervals by two milkers per day showed marked variation of milk yield and fat per cent. Cows and heifers were milked for stated periods by one milker only per day.

Under one milker the average milk yield and fat percentage was higher at the morning milking than at any other. Under two milkers the average milk yield had been higher in the morning, but the fat percentage was lower.

Under one milker there were lower variability coefficients for fat percentage, both at successive milkings, and at corresponding milkings on successive days.

The preference cows show for one of two good milkers is marked and shows itself in a more even distribution of both milk and fat over each of two or three milkings.

K. W. D. CAMPBELL

T. MARCHLEWSKI. **Certain factors determining the hereditary transmission of an abundant milk production in cows.** Paper at Polish Acad. Sci., Cracow, July 7, 1930. (*Nature*, **126**, No. 3191, 1014, December 27, 1930.)

S. W. MEAD and W. M. REGAN. **Deficiencies in rations devoid of roughage for calves. I. The effect of the addition of cod-liver oil and alfalfa ash.** *J. Dairy Sci.* **14**, iv, 283–93, July 1931.

The authors conclude from prolonged experiments on eight animals, that calves can be reared to 19 months of age, and that normal growth can be secured on a ration containing no roughage, providing that cod-liver oil and alfalfa ash are supplied in sufficient quantity. Before the cod-liver oil was given, the animals developed definite symptoms of vitamin A deficiency.

J. GOLDING

N. HANSSON and N. E. OLOFSSON. **Tests of the influence of different fodders on butter consistency.** *Kgl. Landtbruks-Akad. Handl. Tid.* **70**, 621–55, 1931. (*Chem. Abst.* **25**, xvii, 4631, September 10, 1931.)

The consistency of butter is affected by the composition of the fodder and the methods of preparation of the butter. Coarse green fodder, clovers and rich grass give a soft butter of high iodine number. Grass as ensilage or hay loses the property of giving soft butter. Coarse winter fodder gives a hard solid butter with little difference between the effects of hay and straw. Roots and cabbages act like straw. The effect of home grown concentrates depends on fat content. Barley gives a hard butter and peas and beans a hard brittle butter, while oats and mixed grain have a less effect. The effect of purchased concentrates depends on the oil content and the oleic acid content of the oil. A food of high oil content with a high iodine value tends to give a soft butter and a food containing oil of low iodine value gives a hard butter. A winter concentrate should contain 5–6 per cent. oil of an iodine value of 100–110 to give the

proper consistency to winter butter. Soft butters are given by linseed, sunflower, sesame and rapeseed cakes. Hard butters are given by coconut and palm cakes, cakes low in oil and legume meals. Peanut, cotton seed, soya bean and sunflower meal give butter of medium consistency. A cow reacts within 8 days of commencing to feed a new cake but the response as shown by the composition of the butter is variable from cow to cow, as variations in iodine value of 6–7 are given. Butter consistency is greatly influenced by changes in cooling the cream and working. W. L. DAVIES

K. RICHTER, K. E. FERBER and N. ODAISKY. **The effect of feeding raw and steamed potatoes on the yield and fat content of milk.** *Wiss. Arch. Landw.* Abt. B, Tierernähr. Tierzucht, **4**, 695–715, 1931. (*Chem. Abst.* **25**, xii, 3094, June 20, 1931.)

The yield and fat content of milk from cows on a normal diet were compared with those obtained when 12 kg. raw potatoes replaced an equal amount (starch values) of the diet. The fat content did not vary, but the yield of milk decreased 5 per cent. Potatoes were steamed and placed in a silo for 3 months; these potatoes were used in an experiment similar to the one using raw potatoes; the amount of milk decreased 4 per cent., and the fat content dropped from 3·4 to 3·14 per cent. Analysis showed that the steam treatment had no appreciable effect on the chemical composition of the potatoes.

J. GOLDING and A. H. BLISSETT. **Results obtained by feeding small quantities of cows' milk to porkers.** *Pig Breeders' Annual*, 1931–2.

This paper gives the results of three years' work in which 14 separate experiments involving 162 pigs were conducted. In each experiment the pigs were all fed on a similar basal diet, generally accepted as a good ration for porkers. One lot received half a pint of full cream milk daily from the Institute tuberculin tested herd. This milk contained about 1·32 oz. of dry matter. The control lot of pigs received 2 oz. more of the meal mixture to balance the proteins fed in the milk. Although five different rations were fed and although grass, kale and swedes were included, the pigs receiving milk invariably grew more rapidly than the control pigs.

A chart shows that the increase in weight which was only from 1 to 4 per cent. in the summer months, rose to over 10 per cent. in the winter months, giving a good return for the small quantity of milk to which this increase, and improvement in appearance also noted, is attributed. J. GOLDING

A. S. FOOT, J. GOLDING and S. K. KON. **A note on the requirements of the pig for the vitamin-B complex.** *J. Soc. Chem. Ind.* **50**, xxi, 442–3, May 22, 1931.

Four litter-mate pigs weighing 32–34 lb. were divided into two similar lots which were kept in metabolic cages on screens. Lot *A* received a basal diet of polished rice (washed, cooked and dried) light white casein, filter paper pulp and salt mixture. Lot *B* received a similar diet in which 5 per cent. of dried brewers' yeast replaced equivalent protein in the diet. Ten, and later 20 c.c. of cod-liver oil were administered to each pig. Tested on rats, diet *A* was shown to be completely deficient in vitamin-B complex. Diet *B* produced normal growth. The pigs in lot *B* also grew well, and now, after four months on the diet, the sow weighs 163½ lb., and the barrow pig 128½ lb., and they appear normal and healthy. The pigs in lot *A* did not grow at all. Their coats became matted and soiled, and their appetite declined steadily. The barrow pig was killed for histological examination after it had been 11 weeks on the diet without showing definite symptoms of vitamin-B deficiency. The sow pig developed a paralysis of the hind limbs after 12 weeks on the diet. This was not relieved by yeast therapy instituted 48 hours after the onset of symptoms. The pig died of septic pneumonia.

J. M. Evvard, C. C. Culbertson and Q. W. Wallace. **Dried buttermilk for growing and fattening pigs.** *Iowa Agr. Exp. Sta. Bull.* **278**, April 1931.

An experiment is reported in which dried buttermilk alone or mixed in varying proportions with tankage is compared with tankage alone as a supplement to a diet consisting of shelled corn (maize) and salt self-fed to fattening pigs.

The addition of dried buttermilk increased the daily live-weight gains in all cases except one, where the percentage of dried buttermilk in the protein supplement was only 10 per cent.

In this experiment the proteins of dried buttermilk were more efficient than those of tankage in balancing the nutritional deficiencies of corn.

Records of water consumption and body measurements of the pigs are given.

A. S. Foot

DAIRY INDUSTRY.

Amending regulations under the Dairy Industry Act (New Zealand). *New Zealand J. Agric.* **43**, ii, 150, August 1931.

Among the amendments to the Dairy Regulations under the above act gazetted on July 30, 1931, may be mentioned the following:

1. The manufacture and export of other than whole-milk cheese is prohibited.
2. The occupier of a dairy is required to provide an adequate water supply and plant adjacent to his dairy for the purpose of washing and scalding or steaming his dairy utensils.

Other regulations deal with the cooling of milk on the farm, the keeping of cheese in the curing room, the temperature of grading stores and the testing of milk.

J. Golding

Gates. **The Army's milk supply.** *Journal R.A.M.C.* 56, May 1931.

The author discusses the difficulties of the Army's milk supply, particularly in foreign stations and during war.

Two types of dried milk are discussed and compared with condensed milks as now supplied to the services.

The comparison is very much in favour of the dried milk. The author says: "Of the powdered milks those made by the roller process, or better still, if obtainable, those made by the improved roller process are more suitable for military purposes, owing to far better keeping qualities." The short heat treatment involved in the roller process, it is also claimed, does not impair the vitamin content. J. Golding

E. F. Eldridge. **Disposal studies for milk-products waste.** *Engin. News Rec.* **106**, 520–1. (*Exp. Sta. Rec.* **65**, iv, 384, September 1931.)

This paper deals with details of the disposal of diluted dairy waste. J. Golding

O. Koch. **Die Sterilisation der Milchflaschen.** (The sterilisation of milk bottles.) *Milchw. Zbl.* **60**, vii, 102–4, April 15, 1931.

Impressed by the importance laid on the sterilisation of milk bottles after they had been washed, as had been observed during a tour in England, the author later carried out comparative tests, the results of which are recorded in this article. The bottles which had been only washed invariably contained large numbers of bacteria, whilst those that had been steamed for 6–8 minutes after washing were, practically speaking, sterile. These findings have been applied in practice, and suitable steaming chests have been designed. E. R. Hiscox

A. C. Fay. **The preparation, testing and use of chlorine disinfectants.** *Kansas Agr. Exp. Sta.* Circ. 160, July 1931.

A practical guide to the preparation, standardisation and use of hypochlorite disinfectants in the dairy. The methods described are simple, and such as could be applied in a small dairy without the use of elaborate equipment. Suitable dilutions are suggested for various disinfecting purposes, and precautions to be observed in their use are given. E. R. Hiscox

K. Vas. **Das spezifische Gewicht, der Wassergehalt der Bruchkörner und die Rolle des Wärmens (Brennens) bei der Emmentaler Käsebereitung.** (Specific gravity, water content of pieces of cut curd and the part played by heat in the manufacture of Emmenthal cheese.) *Milchw. Forsch.* **11**, iv–v, 519–29, March 1931.

The author points out that accurate knowledge of the properties of the cubes of curd is very important because they regulate the water content of the cheese. Instead of leaving judgment to the senses, as is customary, the author uses a "coagulometer" to measure the resistance to pressure shown by the cubes. In general, this property increases with decreasing water content. On the other hand, when kept pressed together through the agency of the cheese coat, the enclosed pieces of curd may develop great resistance, even if they contain considerable moisture.

A close relationship was found to exist between the specific gravity of the cubes and their water content—increase in the former being a good indicator of decrease in moisture. Determinations of specific gravity may be made by suspending in salt solution or in a mixture of paraffin and carbon tetrachloride.

Experiments on these lines showed that long heating of the cheese curd not only results in a marked decrease in water content but also causes the cubes to stiffen and become less sticky. The importance of these effects lies in the fact that these pieces of curd, when pressed together, form a continuous canal system which regulates the rate at which the enclosed whey can leave the cheese through the interstices. L. A. Allen

W. H. Udy. **Mottled colour in cheese.** *New Zealand J. Agric.* **42**, iv, 244–5, April 1931.

Mottling in annattoed cheese is regarded as being generally due to bacterial reduction, as it occurs most frequently in hot weather. A Gram-negative bacillus has been isolated which bleaches annatto media.

Another important cause of some forms of mottling has been found to be sunlight playing on the annattoed milk in the vat. Hence direct sunlight should not be allowed to shine on the cheese vat.

(This reduction has been shown to be due to the activation of constituents of the milk, especially the olein, which is oxidised while the pigment acts as a hydrogen acceptor. The same phenomenon has been observed in England with various oxidation-reduction potential indicators dissolved in the cheese milk.) J. G. Davis

W. V. Price. **The influence of sweetened, frozen cream on the development of the swell in ice cream.** *J. Dairy Sci.* **14**, iii, 221–8, May 1931.

Fresh pasteurised cream was divided into four batches, the first batch was maintained at 35° F. and was regarded as the control, the second was frozen for 48 hours, the third received an additional 10 per cent. of sucrose by weight, while the fourth received an equivalent weight of invert sugar. The last three batches were frozen at − 15° F. for 48 hours and were then thawed in cold water very slowly. Four mixes were made which differed from each other only in that the cream has received different treatment. The mixes containing frozen, unsweetened cream were slightly

inferior in whipping qualities to the others which behaved almost identically. Similar results were obtained when the cream was frozen at – 15° F. for 14 days.

As a result of further experiments on mixes containing egg-yolk frozen with and without invert sugar, it is suggested that the lecithin of the cream and yolk favours swell development, and that sugar has a beneficial influence on the stability of lecithin during freezing at – 15° F. F. Procter

REVIEWS

Sir William Dampier. **Dairy Research. A Report to the Empire Marketing Board.** Pp. 64. London: H.M.S.O., 1931. 1*s*.

A few days before his death, the first editor of the *Journal of Dairy Research* asked the writer to write a review of Sir William Dampier's monograph, because he was "doubtful of the advisability of its being done by any of us who are actually engaged in research." Elsewhere in his letter he wrote: "I think that few people have realised the magnitude of the problems, that are involved in research in dairying," and he referred to his own brief summary of these on pp. 23–2 of the *Annual Report of the N.I.R.D.* for 1930.

There can be no doubt that what Dr Williams had in mind was the almost inevitable tendency of research workers to over-emphasise the importance of that section of the field in which they work. Sir William's monograph is an excellent corrective to this. The three questions referred to Sir William Dampier by the E.M.B. in 1929 were:

(1) What is dairy research, *i.e.* what is the proper line of division between a specialist dairy research institute and the work of other research institutions in the sphere of nutrition, animal diseases and low temperature research?

(2) What is being done in this field in England, Scotland and elsewhere?

(3) What extensions are contemplated or desirable, and what are the most important scientific and practical problems to which attention should be directed?

Important as questions (1) and (3) were in 1929, they are incomparably more important now, when the problem of getting the best value for the expenditure of limited national funds is more insistent, and there will be no regrets that Sir William was not stampeded into delivering a quicker report, for such a report could not have carried the weight of that now presented.

Should anyone be asked to abstract this report, he would be set an impossible task. The author devotes half his space to a review of dairy research now in progress and the rest to answering the other questions referred to him, which are, in effect, to make recommendations to eliminate undesirable overlapping and to indicate what, in his view, seem the directions in which research is most likely to yield important results in the near future. No dairy researcher can fail to be stimulated by reading this report. The more myopic will be helped to see the whole great field of dairy research in better perspective. G. C. Jones

Ethel Browning. **The Vitamins.** Monographs of the Pickett-Thompson Research Laboratory. Vol. **1**, September 1931. Pp. xxxii + 575. Baillière, Tindall and Cox.

According to the author's introduction, "The original purpose of this monograph was to collect into one volume as much as possible of the existing literature on the subject of Vitamins, and to present to the scientific reader a comprehensive account of the researches which have established the vitamin theory on its present basis...."

The task is an enormous one, and the author has undoubtedly given ample proof

of great perseverance and tireless labour in collecting, reading and attempting to classify nearly 4000 articles. It must unfortunately be said that she has failed in her "endeavour to weave into a complete pattern those threads which have seemed most important to the continuity of the design." An apparent lack of first hand knowledge of the subject and a not less apparent lack of biochemical training on the part of the author have caused Dr Browning's book to be little more than a confused mass of data and references rather than an orderly presentation of facts and theories. The reviewer has noted in the course of one reading and in the text only, not less that 165 errors, some admittedly of a minor nature. Many of these may be laid at the door of faulty proof reading, but it is hard to pass unnoticed scores of more fundamental errors such as the following which illustrate the shortcomings of the book: on p. 67 the author states that "Carotin is an unsaturated hydrocarbon ($C_{40}H_{56}$) with a strong affinity for oxygen, being transformed into colourless xanthophyll." On p. 69, when comparing vitamin A and carotene, the author states "both compounds give a blue colour with antimony trichloride, but with vitamin A the colour is permanent, with carotene it is fleeting." On p. 83 one finds the surprising statement that "Vitamin A is the easiest of the vitamins to preserve." On p. 117 it is said that "the meat residue was treated for extraction of casein," while on p. 133 we learn that "When treated with digitonin, vitamin D forms an insoluble additive compound, and the reaction product is no longer activable." On p. 143 the stock diet of Steenbock is called "diet 2965." The following description of glutathione (pp. 232–3) gives an idea of the chemistry as found in the book: "Glutathion is a dipeptid consisting of a molecule of cystin combined with a molecule of glutamic acid. The formula is a complicated one, the importance being, it appears, the possession of an SH group and the power of reduction of sulphur derivatives, cystin, thioglycocolic and thiolactic acids. The chemical reaction which it undergoes is a to-and-fro one, reduction and oxidation, and it is a typical example of a body which can act in both these ways. The study of its action is indicated in pathological conditions accompanied by disturbance of cellular function." The following heading is found on p. 293: "Vitamin B_2 (Vitamin C_1 of American writers)." Page 299 gives "The aqueduct was completely restored, and all possible sources of contamination were removed, while a number of minute pieces of calcium (previously well washed) were introduced into the reservoir and along the channel." "Cod-liver oil is a preventive both for black tongue and pellagra," p. 312; "the antiscorbutic value of fresh limes is very low—about that of lemon juice," p. 328. On p. 409 we learn of the "increasing tendency to regard vitamins as inorganic chemical bodies," while on p. 426 we are asked to believe that "the *loss of appetite*" (*italics of the reviewer*), "loss of weight and fall of body temperature, which are three of the most characteristic features of vitamin B_1 deficiency, have all been attributed to inanition rather than to a specific effect of vitamin B_1."

Such examples could be greatly multiplied, as nearly every page of the book brings us a fresh surprise. Apart from such errors, it is often evident that Dr Browning does not understand the subject she discusses. To quote only a few instances, this is apparent in the paragraphs on the relation of vitamins to protein intake, pp. 27–9, the discussion of the mechanism of formation of vitamin D on p. 130, and the explanation of refection on p. 427.

The chapter on the quantitative estimation of vitamins, pp. 48–61, is full of inaccuracies and omissions—*e.g.* the rachitogenic diet, diet 2965 of Steenbock, and the simple scurvy-producing diet of the Lister Institute are not mentioned at all. The compositions of standard diets for the production of vitamin A, vitamin B_1 and vitamin B_2 deficiencies are not given and the estimation of vitamin E is dismissed in a few inadequate words.

The extensive tables showing the vitamin content of foodstuffs could have proved

quite useful, but unfortunately here also inaccuracies are found, for example, irradiated yeast is allotted 2 crosses for its vitamin D content while cod-liver oil and irradiated butter are given three where it is known that irradiated yeast is about 75 times as potent as good cod-liver oil.

The extensive bibliography loses much of its value on account of several references being given to the same author and year with no distinguishing marks in the text.

S. K. Kon

W. Clayton. **Colloid aspects of food chemistry and technology.** Pp. viii + 571 (1–446 text, 446–571 glossary, bibliography and index). London: J. and A. Churchill, 1932. 36*s*.

Up to the present no attempt has been made to survey the field of food chemistry with reference to those physico-chemical aspects which come within the range of modern colloid chemistry, and this volume seeks to fill this gap in textbook literature and may be taken as a guide to colloid problems which may be unfamiliar to food chemists who have not specialised in colloid chemistry.

The text of thirteen chapters commences with an historical survey embodying the scope of modern colloid concepts followed by well-arranged comprehensive treatment of the industrial application of these concepts to all branches of food technology including gums, proteins, cereals, milk and its products, sugar fruit preserves, brewing, cold storage of animal and vegetable foods, and sewage. The colloid aspects of nutritional chemistry are also discussed.

Milk and its products are treated fully from the physical and physico-chemical aspects in 87 pages of condensed descriptive material. This section is well supported by references and will be useful for the research worker requiring either condensed information on special physical points or indications as to where the problems have been elaborated in the journals. Special mention must be given to the treatment of milk, cream and butter, as emulsions in which theories on the composition of the fat-globule membrane and the state of water in butter are discussed fully.

Industrial processes such as pasteurisation, homogenisation and cream whipping are included, and the coagulation of milk, especially by rennet action, and the manufacture of the various types of casein are fully described. Other sections include the physical chemistry of cheese, ice cream and milk coagulation in the stomach.

The book can be recommended as a concise source of up-to-date information for the dairy research worker, and to the teacher requiring material for advanced students. The additional advantage of having at hand information on cognate problems in other branches of food chemistry is evident. The bibliography is extensive, references both at the foot of the pages and collected into a separate section (96 pages), being supplied, and a complete author and subject index are also included.

W. L. Davies

STATISTICS

Number of Dairy and Other Cattle.

The following table shows comparative figures of the dairy herd, and of other cattle, in the principal countries within the British Empire in which dairying is of major importance. The figures are in each case the latest available, and are generally of a preliminary nature, but it should be noted that the estimates of livestock relate to different dates in the various countries. It should also be noted that classification of cattle into the dairy herd and other cattle is not a matter of uniform practice throughout the Empire, but the figures give a fairly accurate indication of the extent of the dairy herd and of any change in comparison with the preceding census.

Countries	Year*	Cows and heifers in milk or in calf	Other cattle	Total cattle
		000 head	000 head	000 head
England and Wales	1931	2790	3274	6,064
	1930	2675	3175	5,850
Scotland	1931	454	755	1,209
	1930	453	783	1,236
Northern Ireland	1931	258	422	680
	1930	256	417	673
Irish Free State	1931	1300	2729	4,029
	1930	1311	2727	4,038
Canada	1931	3513	4478	7,991
	1930	3683	5254	8,937
Australia	1929–30	2465	8737	11,202
	1928–29	2467	8834	11,301
New Zealand	1931	1602	2479	4,081
	1930	1441	2329	3,770
Union of South Africa	1930	Not available		10,574†
	1929	Not available		10,518
Kenya	1931	71	166	237‡
	1930	68	159	227

* Estimates relate to June except New Zealand (January 1), South Africa (August 31), Kenya (February 28, 1931 and July 31, 1930); for Australian States estimates refer to December 31 except New South Wales and F.C.T. (June 30 following).

† Including 6,668,000 cattle on farms owned by Europeans.

‡ Relates solely to European-owned cattle; the estimated number of native-owned cattle in Kenya was 4,966,000 on July 31, 1930.

Trade in Dairy Produce.

The following tables give details of the trade in butter and cheese of the principal countries handling these products during 1931, with comparative figures for 1930. The particulars are based on information published by the International Institute of Agriculture, Rome, brought up to date, where necessary, from official sources.

Exports and imports of butter.

Countries	Exports		Imports	
	1931	1930	1931	1930
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Austria	2,862	4,112	1,565	545
Denmark	378,429	372,558	1,596	1,389
Estonia	31,844	31,010	—	—
Irish Free State ...	42,307	58,766	3,324	3,391
Finland	38,367	37,726	—	7
Hungary	4,065	3,430	117	40
Latvia	41,313	40,631	24	49
Lithuania... ...	19,191	16,219	—	—
Netherlands ...	72,660	92,394	8,887	4,396
Norway	1,629	236	379	1,530
Poland	27,470	26,714	31	29
Sweden	43,162	58,857	40	18
U.S.S.R.	68,027	23,198	—	—
Canada	10,681	1,179	2,822	38,605
U.S. America ...	2,004	2,967	1,883	2,471
Argentina ...	47,948	51,156	—	—
India	364	551	344	282
Syria and Lebanon	1,817	2,161	344	172
Australia	208,924	126,411	—	2
New Zealand ...	221,083	208,170	—	—
Importing countries				
Belgium	2,756	2,648	41,562	22,633
Czecho-Slovakia ...	661	694	4,107	714
France	11,036	12,106	40,836	12,921
Germany	269	575	220,950	293,560
Greece	—	—	2,059	1,420
Italy	1,290	1,843	6,188	3,115
Spain	88	161	121	328
Switzerland ...	20	40	23,358	18,794
United Kingdom...	40,228	20,514	903,969	764,019
Ceylon	—	—	642	723
Java and Madura	—	—	7,862*	7,158*
Japan	—	—	231	611
Algeria	66*	73*	4,237*	3,040*
Egypt	44*	42*	1,918*	2,156*
Tunis	9	13	930	829

* From January 1 to November 30 only.

Exports and imports of cheese.

Countries	Exports		Imports	
	1931	1930	1931	1930
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Austria	6,213	4,480	5,792	5,637
Czecho-Slovakia ...	10,981	8,274	3,779	2,963
Denmark	9,423	12,626	604	809
Finland	5,776	4,683	33	35
Italy	89,045	80,976	10,115	12,562
Lithuania... ...	2,546	1,960	11	11
Netherlands ...	190,460	206,739	1,345	1,510
Norway	2,840	1,380	562	750
Poland	2,884	3,267	761	1,974
Switzerland ...	54,307	66,143	8,470	4,237
Yugo-Slavia ...	4,198	4,583	243	300
Canada	84,790	66,955	1,446	1,779
Australia	7,405	7,273	24	150
New Zealand ...	181,891	201,256	4	4
Importing countries				
Belgium	814	875	49,600	51,106
France	33,259	38,921	82,810	65,519
Germany	7,372	5,410	120,404	137,459
Greece	190	302	3,960	2,302
Hungary	110	93	203	386
Irish Free State ...	154*	170*	2,689	2,350
Portugal	—	—	842	1,010
Spain	236	207	3,867	5,836
Sweden	—	—	1,691	1,470
United Kingdom...	7,346	8,931	323,091	348,578
United States ...	1,865	2,127	61,992	68,313
India	7	7	886	1,133
Java and Madura	—	—	1,497*	1,526*
Syria and Lebanon	86	132	708	730
Algeria	157*	196*	10,446*	9,310*
Egypt	57*	60*	6,768*	6,828*
Tunis	24	29	2,033	1,746

* From January 1 to November 30 only.

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BY

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